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THE GENUS CANTHARELLUS IN THE WESTERN UNITED STATES *

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(WITH 13 FIGURES)

Since species of *Cantharellus* are conspicuous elements of the agaric flora of our western states, and because several are important edible species, it appeared desirable to bring our information on them up to date. We ourselves did not realize the full extent of the need for a comprehensive treatment of this genus for the region until several seasons of concentration had served to focus our attention on a number of problems. In the past the difficulties of working with species of *Cantharellus* in this area were twofold. Reports of the species were scattered in the literature and contained very little essential information. No work was available which focused attention on the problems peculiar to the region. It now appears that a number of conspicuous elements in the flora were undescribed.

In keeping with the present day trend in the classification of the gill fungi, we recognize the family Cantharellaceae as a family distinct from the true gill fungi, and one most closely related to the Clavariaceae. The characters of the basidia indicate this relationship. In both groups the basidia bear from two to eight spores and the spindles of the dividing nuclei are arranged more or less parallel to the longitudinal axis of the basidium instead of at right angles to it. There is also considerable intergradation in the type

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of fruiting body produced, the configuration of the hymenium, and the type of spores. The following key to the genera of the family is given as an aid to those whose interest extends beyond the scope of this paper. In this key we have not tried to delimit the Clavariaceae sharply from the Cantharellaceae. Most of the intergrading species probably are best placed in *Craterellus*.

KEY TO THE GENERA OF THE CANTHARELLACEAE

A. Fruiting body typically fleshy and centrally stipitate or stipe eccentric but well developed (in some the stipe and pileus not clearly distinct, the fruiting body then being in the form of a trumpet or funnel).
  1. Hymenium smooth or nearly so .... *Craterellus*
  1. Hymenium in the form of distinct radiating ridges or folds or as obtuse forked lamellae .... *Cantharellus*

A. Fruiting body very thin and delicate; stipe if present very small and rudimentary.
  1. Spores hyaline .... *Leptotus*
  1. Spores tawny to incarnate .... *Arrhenia*

CANTHARELLUS Fries, 1821

Fruiting body fleshy to moderately tough, sometimes woody when dried, pileate to tubular or trumpet-shaped, pileus and stipe not always clearly distinct; hymenium borne on the under side of the pileus and upper portion of the stipe, usually in the form of narrow gill-like folds which branch dichotomously or anastomose and have obtuse edges, usually strongly intervenose and in some species occasionally somewhat poroid from the pronounced cross-veins, often with a waxy luster; basidia typically elongated and with flexuous pedicels, two to eight spored, reaction in KOH typically negative in all but the first two sections. (In *C. Underwoodii* it is olive; in *C. pseudoclavatus* and *C. clavatus* it is dull brown or orange-brown respectively.) Spores white to yellowish to pale alutaceous in deposits, smooth or with the outer wall slightly wrinkled or pitted, nonamyloid (yellow to rusty brown in iodine solution); hyphae of the gill and pileus trama scarcely distinguishable in shape or form from each other, typically hyaline in KOH, but in one group with a slight coloration; clamp connections either present or absent depending on the species; cystidia typically absent.

The present study brought out some interesting groupings of species within the genus, and as a result of it the following sections are recognized:

SECTION I: **Polyozellus.** The single species in this section was made the type of the genus *Polyozellus* by Murrill. However, we define the section as characterized by the small, roughened, hyaline spores and the color change of the flesh in KOH rather than by the characters used by Murrill in erecting it as a genus.

SECTION II: **Gomphus.** This section contains three species, *C. clavatus, C. pseudoclavatus* and *C. brevipes*. This group has been recognized as a genus, *Neurophyllum* Patouillard and *Gomphus* S. F. Gray, both based on *C. clavatus*. In our estimation this is not an acceptable arrangement because it is difficult to find any characters other than general aspect which will distinguish between *Gomphus* and Eu-Cantharellus. We define the section Gomphus as containing species similar in appearance to *C. clavatus*. It is interesting to note in this group that clamp connections are abundant on the type species, *C. clavatus,* which is circumpolar in distribution, but absent on the other two which appear to be relatively local segregates of it. In our estimation such occurrences as this are sufficient reason for not placing great emphasis on the presence or absence of clamp connections in erecting new genera. For here in *Cantharellus* we have groups in which *so far* clamps have not been found (Section Excavatus), one section in which the central species has them but the segregates do not (Section Gomphus), and two sections where, in so far as we know them, clamps are regularly present.

SECTION III: **Eu-Cantharellus.** The type of this section as well as for the genus is *C. cibarius*. In our region the section is represented by only two species, the type and *C. subalbidus*. The section may be defined as containing fleshy species with essentially smooth pilei, in other words fungi resembling *C. cibarius* in general aspect. In the Western United States *C. cibarius* is extremely variable, but we have not attempted to classify variants below the species level because of the lack of any constant characters. Clamp connections are found on both species. The origin of this section appears to us to be in Europe for it is there that one finds the largest number of species described which are obviously closely related to *C. cibarius,* and the latter, itself, is very common. There have been a number of varieties of *C. cibarius*

described from Europe which, if the descriptions are correct, appear to be autonomous species.

SECTION IV: **Excavatus.** The characters of this section are the innately fibrillose scaly to squamose pileus which in most species becomes hollowed out to produce a deep central depression or becomes hollow almost to the base of the stipe. The type of this section, logically, is *C. floccosus* since it appears to be the one from which the others have been differentiated. The section contains *C. Kauffmanii, C. floccosus, C. Bonarii* and *C. Wilkinsae*. In the order named these species show a progressive change from the very fleshy-brittle type as represented by *C. Kauffmanii* to the semi-woody condition found in *C. Wilkinsae*. In addition to the character previously mentioned the section is characterized by the spores, which are rusty brown in iodine, and the lack of clamp connections on the hyphae of the fruiting bodies. Although *C. floccosus* was originally described from the eastern seaboard, it appears clear to us that the center of distribution for the section is the region under consideration here. As evidence for this statement we cite the number of species known from the area (it is larger than that known for any other region), the abundance of most of the species and the great variation observed in the type, *C. floccosus*.

SECTION V: **Tubaeformis.** We regard this as a group in which the species are not yet properly classified and in which the nomenclature remains to be worked out. We believe there is little to be gained here by nomenclatorial studies until such time as workable species concepts become established. In our opinion this section promises to be one of the largest in the genus and our information so far indicates northeastern North America and the Great Lakes Region as being the critical area. It may be defined as containing species similar in general aspect to *C. tubaeformis*.

EXCLUDED SPECIES: As limited here *C. umbonatus* with amyloid spores, *C. olidus* and *C. aurantiacus* are excluded from *Cantharellus*. *C. umbonatus* is now the type of the genus *Cantharellula* Singer and the others are placed in *Clitocybe* by most investigators since they are regarded as true gill fungi. All of these species occur in our region and since they are likely to be sought for in *Cantharellus*, they have been included in our key to species.

A number of apparently very rare and obviously poorly known species have been reported from the area. These have been omitted from this work. For instance *C. albidus* Fr. has been reported, but the only specimens examined turned out to be *Omphalina umbellifera*.

We have also refrained from citing long lists of collections. The specimens upon which this paper is based will be found in the Herbarium of the University of Michigan, Herbarium of the University of California, and in the Morse Collection, Bureau of Plant Industry, Beltsville, Maryland. The color names in parentheses are taken from *Color Standards and Color Nomenclature,* by R. Ridgway, 1912.

It is a pleasure to acknowledge the assistance of our colleagues who have aided this investigation by collecting material or making available material deposited in the herbaria of their respective institutions. We are indebted to Dr. H. D. House, New York State Museum, Albany, N. Y., for the opportunity of examining certain of Peck's types. Dr. Lee Bonar of the University of California made available to us the critical collections of *Cantharellus* at that institution. To the others who likewise aided us in our project we extend our sincere thanks.

KEY TO THE WESTERN SPECIES OF CANTHARELLUS

1. Spores amyloid; pileus typically grayish to violet-gray; gills close, white and staining yellowish to reddish when bruised. *Cantharellula umbonata* (Fr.) Singer
1. Spores not amyloid.......2
2. Lamellae close to crowded.......3
2. Lamellae distant to subdistant or merely in the form of ridges (if close not bright orange and flesh not with an odor of cinnamon candy).......4
3. Gills bright orange; spores $5–7 \times 3–4\ \mu$; odor not fragrant. *Clitocybe aurantiaca* (Fr.) Studer
3. Gills pale buff; spores $3–4.5 \times 2.5–3\ \mu$; odor typically reminding one of cinnamon candy.......*Clitocybe olida* (Quél.) Kon.
4. Flesh of pileus dark blackish green to olivaceous in dilute KOH. 1. *Cantharellus multiplex*
4. Flesh of pileus not as above when treated with KOH.......5
5. Surface of pileus not regularly breaking up into coarse recurved fibrillose scales.......6
5. Surface of pileus soon broken up into coarse scales or cap splitting into a number of segments.......11
6. Hymenium typically tinged vinaceous, purplish or smoky violaceous; carpophores typically large (3–10 cm.).......7

6. Hymenium white to yellow or orange, if colors clouded with gray the fruiting bodies small (1–3 cm. or up to 5 cm.) 8
7. Spores smooth; hymenium sordid bister in KOH.... 2. *C. pseudoclavatus*
7. Spores roughened; hymenium orange to orange-brown revived in KOH. 3. *C. clavatus*
8. Pileus typically large, 3–10 cm. 10
8. Pileus typically small and perforated, grayish to sordid yellowish brown.. 9
9. Spores white to whitish in deposits .... 10. *C. tubaeformis*
9. Spores ochraceous to ochraceous salmon in deposits. 11. *C. infundibuliformis*
10. Pileus gills and stipe pallid, spore deposit whitish .... 4. *C. subalbidus*
10. Pileus gills and stipe yellow to orange; spore deposit "light cinnamon buff" (yellow) .... 5. *C. cibarius*
11. Pileus very brittle, never with orange or reddish hues in the scales, hymenium becoming merulioid .... 6. *C. Kauffmanii*
11. Not with above combination of characters 12
12. Fruiting bodies often cespitose or several arising from a common stipe, numerous small aborted carpophores often present in clusters. 8. *C. Bonarii*
12. Fruiting bodies typically gregarious to scattered 13
13. Spores smooth; cap splitting into distinct segments; texture semi-woody .... 9. *C. Wilkinsae*
13. Spores with slightly wrinkled exospore; cap not splitting into distinct segments (though sometimes scales may be very coarse); scales orange to bright yellowish orange .... 7. *C. floccosus*

1. CANTHARELLUS MULTIPLEX Underwood, Bull. Torrey Club **26**: 254. 1899.

Polyozellus multiplex Murrill, North American Flora **9**: 171. 1910.

Craterellus multiplex Shope, Mycologia **30**: 373. 1938.

ILLUSTRATIONS: Bull. Torrey Club **26**, p. 254; Mycologia **29**, p. 287, fig. 1; *Ibid*. **30**, p. 373, fig. 1.

FIG. 1.

Fructifications 6–15 cm. high and up to 1 meter in diam., consisting of a mass of small to moderately large fan-shaped to spathulate pilei 2–5 × 3–10 × 0.1–0.3 cm., in robust carpophores the pilei at times somewhat funnel-shaped, surface indistinctly concentrically zoned with alternate zones of tomentum, or merely unpolished and somewhat roughened and then scarcely zonate, color glaucous violaceous but soon violaceous black or in age somewhat lead-colored, the margin pale glaucous violet to whitish and incurved when young, at times the margin undulating or lobed and usually pubescent at first; flesh rather soft and watery, brittle, dark violet-black, azonate, odor faintly pungent, taste mild, dark

blackish green to olivaceous when treated with KOH; hymenium glaucous violaceous at first ("deep violet plumbeous"), in oldest portion tinged "cinnamon buff," in the form of radiating folds or ridges, the ridges frequently forking or anastomosing or at times giving rise to an almost poroid surface, merely tuberculate to slightly wrinkled where decurrent on the stipe, nearly smooth near cap margin; stipe 3–5 cm. long, 10–20 mm. thick, variously grown together near base or apex or fused in midportion, often irregularly compound, solid, dark violaceous black, fibrous, brittle, slightly roughened and upper part covered by the decurrent hymenium.

Spores white in deposits, broadly elliptic to spheric in outline, tuberculate to angular-tuberculate, $4\text{–}6 \times 4\text{–}6\ \mu$, hyaline to faintly yellowish in iodine; basidia $32\text{–}38 \times 5\text{–}6\ \mu$, four-spored, yellowish in iodine, faintly olivaceous in KOH (very likely from pigment diffusing from the flesh); cystidia imbedded and in the form of narrow somewhat contorted filaments $28\text{–}40 \times 3\text{–}4\ \mu$ (readily demonstrated in sections crushed slightly and stained with phloxine); flesh of interwoven non-gelatinous hyphae with a dark bluish black incrusting pigment on the walls which dissolves into a green solution in KOH; clamp connections present on the basidia.

HABIT, HABITAT AND DISTRIBUTION: It occurs in great clusters or compound clusters on the humus under conifers in the fall or late summer. Material from Union Pass, Pitt Island, British Columbia (McCabe, Sept. 27, 1938), Mt. Rainier National Park, Carbon River Area (Leverett & Richards, Sept. 1930), and Mt. Hood, Oregon (*S–19275* & *24297*) has been studied.

DISCUSSION: It is known from scattered localities across the continent. The collections so far reported for North America, and verified by an examination of material, clearly indicate that the species is northern or alpine in distribution and rare. The white, angular-tuberculate spores and the dark bluish colors in conjunction with the KOH reaction of both fresh and dried material clearly distinguish this fungus from any other known member of the Cantharellaceae. To us the relationships appear to be with *C. clavatus* though it varies more toward *Craterellus*. It seems best, however, to classify it on the basis of the configuration of the hymenium which is cantharelloid in mature fruiting bodies. The spores are unusual for the genus but in our estimation do not warrant excluding the species.

FIG. 1. *Cantharellus multiplex.* ×1.

2. **Cantharellus pseudoclavatus** Smith sp. nov.

Pileus 3–8 (10) cm. latus, planus demum irregulare infundibuliformis, ad marginem undulatus, siccus, subfibrillosus, avellaneus vel sordide alutaceus, ad marginem subpurpureus; lamellae venosae, angustae, decurrentes, subpurpureae; stipes 2–4 cm. longus, 1–2 cm. crassus, solidus, subpurpureus, intus pallidus; sporae 9–12 × 5–6.5 μ, leves, subochraceae.

Pileus 3–8 (10) cm. broad, flat on top and not truly distinct from stipe when young, the margin soon spreading and becoming uplifted and often developing on one side only, pileus finally flabelliform to funnel-shaped, the margin usually very wavy and lobed in age, surface dry and unpolished or in age appressed fibrillose with the fibrils arranged in fascicles at times, color near "vinaceous buff," "cinnamon buff" or "avellaneous" when young, and often with a tinge of purplish pervading along the margin, finally sordid alutaceous but becoming drab in drying; flesh thick in the disc but thin in the extended margin, white to whitish, odor not distinctive, taste not recorded; hymenium variable in form and color, more or less gill-like with the radiating folds moderately close to distinct, narrow and connected by numerous cross-veins to such an extent that the appearance in old caps may be almost poroid, decurrent down almost to the base of the stipe, color when young purplish-vinaceous (colors not matched), in age dusted with the spores and with an ochraceous sheen; stipe 2–4 cm. long, 1–2 cm. thick, solid, pallid within, enlarged upward, surface more or less colored like the cap except for the white mycelioid base, unpolished to tomentose.

Spores yellow in deposits, 9–12 × 5–6.5 μ, subellipsoid, smooth, nonamyloid (pale yellow in iodine), thin-walled and nearly hyaline in KOH, content often appearing amorphous and wrinkled when first revived in KOH; basidia four- to eight-spored, 50–80 × 9–11 (12) μ, often flexuous, when revived in KOH the hymenium appearing sordid brown (pale bister), individual basidia nearly hyaline but with highly refractive amorphous-appearing content; cystidia none; gill trama interwoven, paler than basidia in KOH but thick sections bister like the hymenium to the naked eye, no clamp connections present even at base of basidia, hyphae 5–12 μ in diam. and thin-walled; pileus trama similar to gill trama, surface layer merely more compactly interwoven.

HABIT, HABITAT AND DISTRIBUTION: In this region it is known from one fruiting body found in a mixed woods near the Siskiyou Fork of the Smith River, northern California. In Michigan it has been found in both Washtenaw and Oakland counties in oak-hickory forests. It occurs in clusters much like *C. clavatus*. The

Michigan collections were made on Aug. 8 (*S–6877*) and Aug. 9 (*S–6916.* **Type**), 1937. The California material was found late in November, 1937. It was mistaken for *C. clavatus* in the field and not critically studied. The description has been drawn from the Michigan collections.

DISCUSSION: This species is so like *C. clavatus* in appearance that the senior author was completely deceived by it and discovered its distinctive features only after a routine check in the course of preparing material for accessioning in the herbarium. Microscopically the smooth, broadly ellipsoid spores, lack of clamp connections and reaction of the hymenium and flesh of the pileus and gills in KOH sharply distinguish it. Comments on its abundance and distribution are hardly appropriate at present.

Since *Cantharellus brevipes* also has the aspect of *C. clavatus* it had to be considered in connection with *C. pseudoclavatus.* Through the courtesy of Dr. H. D. House, the type has been examined and the following observations made: The spores are nearly hyaline in KOH. The tinge of color visible was in groups of spores adhering along the hymenium. They measure (12.5) 13–16 × 5.5–7 μ, and are nearly oblong in face view and in side view have a distinct suprahilar depression. The exospore is wrinkled as in *C. clavatus.* The spores, in fact, are similar to those of the European species in shape and markings but differ in length and in their slightly more dilute coloration. The basidia measure 40–50 × 7–8 μ and both two-spored and four-spored individuals were seen. However, the sterigmata are fine and difficult to observe. The color reaction in KOH of the basidia and subhymenium is merely sordid yellowish to brownish and not very distinctive. The colored zone is not sharply delimited as in *C. clavatus,* and to this extent as well as in the darker reaction of the basidia of the latter, the KOH reaction is an aid to their recognition. Cystidia or at least what appear to be sterile filaments are present in the hymenium but in the type it was not possible to be certain that these were not immature basidia. This point needs further clarification. The hyphae of the flesh are very intricately interwoven and 2–5 μ in diam. No clamp connections were found. In *C. clavatus* the hyphae are 5–10 μ in diam. and clamp connections are abundant.

Since *C. brevipes* has been considered to be the same as *C. floccosus* by Murrill and *C. clavatus* by Harper, a critical comparison with them is necessary. Since Peck described the cap of *C. brevipes* as glabrous and alutaceous, and the gills as pale umber tinged with lilac, and since the type seems to agree in the main with the description on these points, there is no reason whatever for considering *C. floccosus* further. Peck's suggestion of such a relationship was obviously based on the stature of the fruiting body and the configuration of the hymenium. *C. brevipes* can be distinguished at once from *C. pseudoclavatus* by its rough spores which measure (12.5) 13–16 × 5–6 μ. From *C. clavatus* it is distinguished by the narrow hyphae of the fruiting body and their lack of clamp connections. The spore size may also be significant but there is likely to be considerable variation in this character. There is the possibility that other differences will also be found, such as the color reaction in KOH when it can be definitely established on the basis of better material, and certain macroscopic characters, but the latter need to be restudied from fresh material.

C. mexicanus Fr. is described as having a grayish fuscous pileus, 3–4 cm. broad, and gills somewhat the color of *C. clavatus* but is said to differ in its well-formed gills, a character which relates it to *C. cibarius*. Two varieties of *C. cibarius*, var. *neglectus* Souché and var. *janthinoxanthus* Maire, have been described with grayish-violet or grayish lilac hymenium. The latter may possibly be the same as *C. pseudoclavatus* since its spores are 11–12 × 7–8 μ and smooth, but this is a question which cannot be answered at present. Maire placed his fungus in *C. cibarius* whereas I mistook the American species for *C. clavatus*—a fact which indicates that the plants must have quite a different aspect when fresh.

3. CANTHARELLUS CLAVATUS Fries, Syst. Myc. 1: 322. 1821.
Merulius clavatus Secretan, Mycogr. Suisse 11: 47. 1833.
Craterellus clavatus Fries, Epicr. Syst. Myc. p. 533. 1838.
Neurophyllum clavatum Patouillard, Tab. An. Fung. fasc. 5. p. 93, no. 434. 1886.
Craterellus carneus Saccardo, Fl. Ital. Crypt. fasc. 15, p. 1135. 1916.

Gomphus clavatus S. F. Gray, Nat. Arr. Brit. Pl. **1**: 638. 1821.

Gomphus truncatus Persoon, Myc. Eur. **2**: 9. 1825.

ILLUSTRATIONS: Kauffman, Ag. Mich. vol. 2, pl. II, upper figs.; Kauffman, Pap. Mich. Acad. Sci. **5**, pl. V (as *Cantharellus multiplex*); Harper, E. T., Mycologia **6**, pls. 93 & 94 (excellent).

FIG. 2.

Pileus (3) 5–10 (15) cm. broad, at first scarcely differentiated from the stipe (the fruiting bodies resembling truncate clubs), soon the margin spreading and frequently developing almost entirely on one side, frequently becoming broadly funnel-shaped from the uplifted margin or flabelliform, the margin usually extensively lobed or sinuate in age, the surface dry and glabrous, unpolished to velvety, in age at times minutely scaly, color at first dull vinaceous to purplish but soon fading to sordid brown ("dark purple drab" when young), soon fading through "light russet vinaceous" or "russet vinaceous" to "avellaneous" and finally "clay color" to "tawny olive"; flesh thick in the disc but thin (5 mm. $\pm$) in the extended margin, whitish to pale buff ("cinnamon buff"), odor and taste mild; lamellae or hymenium variable in color but usually tinged purple or vinaceous ("purplish lilac" to "purplish vinaceous" and fading to near "avellaneous" or "light russet vinaceous"), formed of numerous low, crowded, frequently forked or anastomosing ridges and with numerous thick veins connecting the ridges, at times almost poroid in appearance, decurrent almost to the base of the stipe; stipe frequently compound, 4–10 cm. long, 0.8–3 cm. thick below and expanding into the pileus, sometimes many fused at the base into a large fleshy mass, solid, becoming hollow, whitish below from a thin mycelioid covering, purplish drab above.

Spores narrowly ellipsoid, 10–12 (13) $\times$ 5–6 μ, pale alutaceous in deposits, outer wall somewhat roughened, yellowish in iodine; basidia 65–80 (90) $\times$ 7–9 μ, four-spored, hymenium dull orange to orange-brown when revived in KOH; cystidia none seen; gill trama poorly developed, at first the portion near the subhymenium yellowish in KOH but soon fading and hyaline throughout; pileus trama with a cuticle of upright cells 40–80 $\times$ 2.5–6 μ, brownish in mass when revived in KOH (apparently from the slightly colored walls), nearly hyaline when isolated, forming a very compact turf, hyphae beneath hyaline and compactly interwoven, clamp connections abundant, flesh proper hyaline in KOH.

HABIT, HABITAT AND DISTRIBUTION: Occasionally gregarious but usually cespitose or in compound clusters which may even

FIG. 2. *C. clavatus.* × 1.

occur in arcs or "fairy rings." It is typically on humus though often near very decayed logs, and is most abundant under conifers. It is a common species in northern California, Oregon and Washington in the fall, and occurs at all elevations up to near timber line. During the dry season of 1946 it was very abundant in the vicinity of Wemme, Oregon. We would expect it to be common in British Columbia also.

DISCUSSION: This is a very easily recognized species which one should not confuse with any others save *C. brevipes* and *C. pseudoclavatus*. The purplish to vinaceous tinge in the hymenium is characteristic of all three. The reaction of thin sections of the hymenium in KOH will at once distinguish this species from the other two. In *C. clavatus* the hymenium appears orange to orange-brown and the flesh proper is hyaline. In *C. brevipes* the KOH reaction does not appear to be distinctive and in *C. pseudoclavatus* the hymenium and the flesh become very sordid brown (bister). For further comments see discussion of *C. pseudoclavatus*.

4. **Cantharellus subalbidus** sp. nov. (FIG. 3)

Pileus 5–10 (14) cm. latus, subplanus, ad marginem demum irregulare lobatus, subtomentosus, siccus, albidus vel subalbidus; lamellae angustae, decurrentes, venosae, pallidae, demum luteo-maculatae; stipes 2–4 (5) cm. longus, 1–3 cm. crassus, sursum expansus, albidus demum luteo-maculatus; sporae 7–9 × 5–5.5 μ, leves, albidae.

Pileus 5–10 (14) cm. broad, at first plane or with a decurved margin, soon the margin elevated to somewhat recurved and becoming very irregularly lobed or wavy, in age broadly depressed to subinfundibuliform and quite irregular in shape, surface felty-fibrillose to subtomentose, smooth or in age areolate-scaly, typically dry and unpolished, often very uneven, white to whitish over all, becoming pallid buff when water-soaked and sordid yellow where handled; flesh thick, firm, fibrous, white with a tendency to stain yellow where bruised, odor and taste not distinctive; lamellae close and narrow, long-decurrent, variously forked or anastomosing and strongly veined, white to grayish white but becoming cream-colored and staining yellow to orange when bruised, edges obtuse and even; stipe 2–4 (5) cm. long, 1–3 cm. at base, flaring upward and indistinct from pileus (gills decurrent almost to base), solid, white and fibrous within, surface white and unpolished but stain-

ing yellow to orange when bruised, finally discoloring to sordid brown.

Spores white in deposits, 7–9 × 5–5.5 μ, ellipsoid to broadly ellipsoid, smooth, yellow in iodine; basidia 62–80 × 8.5–10 μ, narrowly clavate, hyaline in KOH but filled with many small oil globules, four to six-spored; pleurocystidia and cheilocystidia none seen; gill trama of loosely interwoven hyaline hyphae, the cells hyaline in KOH and usually filled with many oil drops, thin-walled, 5–8 μ in diam., regularly with clamp connections at cross walls, flexuous and often widened near cross walls; pileus trama homogeneous, the surface of more compactly interwoven cells than the tramal body but of the same type and similar to or slightly broader and more irregular than those of the gill trama.

HABIT, HABITAT AND DISTRIBUTION: Single to gregarious under conifers, particularly Douglas fir, Washington, Oregon and California. It fruits during the fall and winter rainy season and is often abundant.

DISCUSSION: For years this fungus has passed as a white form of *C. cibarius* in this region, but a critical study of it in the Mt. Hood area in 1944 brought out certain facts which indicate that the plant deserves to be ranked as an autonomous species. These observations were verified during the season of 1946. During the latter season Miss McKenny of Olympia, Washington, also brought the species to our attention and commented that it was abundant in her collecting area. We regard the difference in the color of the spore deposit between *C. subalbidus* and *C. cibarius* as fundamental. It is white in the former and "light pinkish cinnamon" in the latter. Fresh prints taken simultaneously were compared under identical light conditions (see collections *S–20030* & *S–20031,* Univ. of Mich. Herb.). The decidedly paler color of the fruiting body is a second constant difference which has always proven to be very reliable in the field unless one chanced upon very old faded *C. cibarius* in exposed places. The pronounced fragrant odor of *C. cibarius* is not present in *C. subalbidus* as far as our specimens to date are concerned.

C. albidus Fr. as described by some European authors appears to have essentially the same color and color changes as *C. subalbidus,* but is a much slenderer plant and according to Ricken has small spores 4–5 × 3 μ. Rea gives the spore size as 6–7 × 4–5 μ.

Inasmuch as accurate data on the color of the spore deposit is lacking on the European species no comparison can be made on that character. Ricken speaks of a white form of *C. cibarius,* but gives no data on the color of the spore deposit. He clearly distinguished between it and *C. albidus.* It is possible that *C. subalbidus* occurs in Europe, but this needs to be verified by a critical study of fresh material.

5. CANTHARELLUS CIBARIUS Fries, Syst. Myc. **1**: 318. 1821 (FIG. 4).

Chanterel Chantarellus Murrill, North Amer. Flora **9**: 169. 1910.

Pileus 4–10 (15) cm. broad, nearly flat and with an inrolled margin when young, margin spreading or becoming uplifted in age and then cap broadly depressed to broadly funnel-shaped, usually wavy or lobed along the margin, surface dry and at first covered by a thin coating of pallid, fine, matted fibrils giving it a canescent appearance, appearing moist in age at times, margin finely pubescent, color pale yellowish young ("cinnamon buff") becoming bright yellow to orange ("antimony yellow" to "deep chrome") in age, finally fading to sordid buff; flesh rather thick and pliant, 1 cm. ± thick near the stipe, whitish or pale yellowish near cap surface, unchanging when bruised, odor fragrant or lacking, or when caps are dried becoming very pronounced, taste mild to slightly peppery; lamellae decurrent, fold-like, dichotomously forked, very narrow, often intervenose, "buff yellow" to "orange-buff" (usually a paler yellow or orange than the pileus); stipe 4–8 cm. long, 8–18 mm. thick, solid, fleshy, whitish within, surface at first finely pruinose-tomentose and concolorous with the gills, in age glabrescent and pallid or faintly yellowish, base often staining sordid orange where bruised.

Spores yellowish ("light pinkish cinnamon") in deposits, 7–9 × 4–5 μ, ellipsoid, smooth, not amyloid; basidia 50–70 × 6–8 μ, very narrowly clavate, mostly four-spored; cheilocystidia and pleurocystidia not seen; gill trama of interwoven, narrow, equal hyphae 3–5 μ in diam. and bearing clamp connections; pileus trama homogeneous, the hyphae near the surface intricately interwoven, clamp connections abundant.

HABIT, HABITAT AND DISTRIBUTION: Single to gregarious in conifer and hardwood forests. It is common throughout the area during the rainy season. In the summer it can frequently be

FIG. 3. *C. subalbidus.* ×1.

found around the edges of bogs and beaver ponds high in the mountains.

DISCUSSION: This species is so well known that comments about it may seem superfluous. However, the variations which occur in this region have not been given sufficient emphasis in the literature, and one who knows the fungus from our eastern states or from the illustrations in the European literature might easily fail to recognize it here. The carpophores vary tremendously in size and the degree to which the margin of the cap becomes wavy or lobed. Some who first chance on these variations without having previously collected the fungus in quantity in this area are likely to consider them as taxonomically distinct forms, varieties or even species. We do not designate them as taxonomic units at any level because of the numerous intergradations which we have observed. According to our observations the carpophores are relatively persistent and continue to enlarge as long as the weather is favorable and food is available. Material from under hemlock on Mt. Baker in Washington was indistinguishable from material collected under oak near Ann Arbor, and so it does not appear that there is any significant difference between the species as it occurs under conifers and under hardwoods. Since weather conditions along our west coast are often favorable for the prolonged growth of fruiting bodies, it is not surprising to find exceptionally large carpophores here, and in eastern United States, where, because of less prolonged periods favorable to growth and preservation of the fruiting bodies, extreme variations are not as frequently realized.

The form illustrated (FIG. 5) was "light vinaceous cinnamon" and the disc "pinkish cinnamon" when the caps were very fresh. This was caused by a fine pruinose-pubescent covering. The pilei were 3–8 cm. broad but occurred in masses 12–15 cm. broad. In some respects this variant approaches *C. amethysteus* Quél., but its spores measured only 7–9 μ long. It is one of the extreme variations encountered in the Mt. Hood area.

No attempt has been made here to list the numerous illustrations of this species in the literature. Nearly every popular mushroom book and regional flora has a picture of it.

FIG. 4. *Cantharellus cibarius.* ×1.

6. **Cantharellus Kauffmanii** Smith sp. nov. (FIGS. 5 & 6)

Pileus (4) 10–20 (35) cm. latus, planus demum late infundibuliformis, siccus, squamosus, alutaceus; lamellae luteae demum pallide alutaceae, plicosae et ramosae, decurrentes, angustae; stipes (3) 8–15 (40) cm. longus, 2–4 (6) cm. crassus, sursum expansus, solidus, firmus; sporae 12–15 × 5–6.5 μ, pallide ochraceae.

Pileus (4) 10–20 (35) cm. broad, flat with a decurved margin when very young, and then solid throughout, very soon splitting downward into the disc to form more or less rectangular segments, finally expanding to broadly vase-shaped and the columnar segments becoming separated and curved in toward the center of the disc to form long innate scales, the scales in the disc becoming worn away leaving a hollow which projects deeper and deeper into the stipe, scales continuing to form near margin as growth progresses and remaining for a time as coarse recurved scales, in age surface along the extreme margin merely split into segments which do not recurve, color of scales "clay color" to "tawny olive," flesh between them when visible whitish; flesh thick, white, firm, unchanging when cut or bruised, taste mild, odor sharp and penetrating but often absent in old caps; lamellae (hymenium) "picric yellow" young, "pinkish buff" in age, in young caps staining vinaceous brown when bruised, in the form of radiating folds at first but in age becoming merulioid with folds going in all directions and forming broad pit-like areas, very narrow, decurrent on the stipe; stipe (3) 8–15 (40) cm. long, 2–4 (6) cm. thick at point where hymenium begins, equal or narrowed downward, solid at first, becoming hollow from apex downward, whitish at first, white within, very firm and hard, often with a long prolongation extending down into the humus for long distances but not in the form of a true pseudorhiza, hymenium often descending unequal distances giving an irregular color pattern.

Spores pale ochraceous in thin deposits, 12–15 × 5–7 μ, narrowly subelliptic and in side view with a suprahilar depression, exospore slightly wrinkled, nonamyloid (rusty brown in iodine); basidia 60–80 × 10–13 μ, two- to four-spored, yellow in iodine, clavate and often with flexuous pedicels; cystidia none seen or represented by slender filaments which could be young basidia; gill trama and pileus trama of intricately interwoven hyaline hyphae lacking clamp connections and measuring 6–10 μ in diam., yellow in iodine, surface hyphae yellowish in KOH but thin-walled and smooth.

HABIT, HABITAT AND DISTRIBUTION: Subcespitose to gregarious or occasionally singly on rich humus in conifer forests. It has

FIG. 5. *Cantharellus Kauffmanii.* × ⅞.

been collected in the Mt. Baker Recreation Area, the Olympic National Park, and on Mt. Hood in Oregon. Kauffman collected it at Lake Quinault, Washington, Oct. 4, 1935. The above

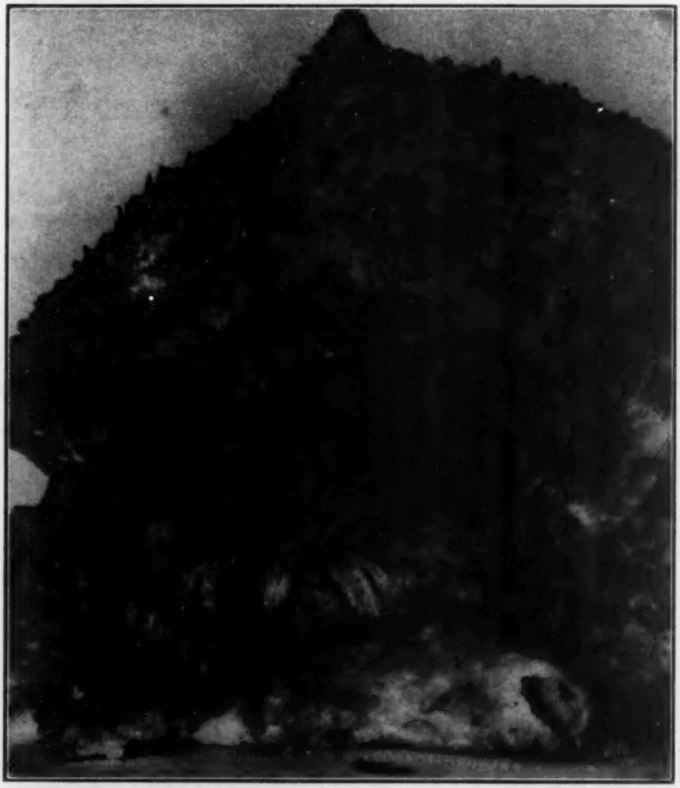

FIG. 6. *Cantharellus Kauffmanii* cap seen from above. × 1.

description was drawn from collection *16156–S* from near Shuksan Inn, near Mt. Baker, Washington, and is designated as the type. During warm wet seasons the species is not infrequently found in the heavy conifer forests where there is a great accumulation of humus.

DISCUSSION: This is the largest *Cantharellus* known in the area if only single carpophores, not clumps, are considered, but it is not exactly a beautiful or graceful species as found in the fully matured state. It is, of course, most closely related to *C. floccosus* by virtue of the coarsely scaly pileus and the stipe which is solid at first but gradually becomes hollowed by the breaking up of the interior into scales. Occasional collections are found of fruiting bodies arrested in their development (typically small and often worm-eaten) in which the stipe remains solid and the cap is flat and covered with coarse scales. The same situation has been observed on retarded fruiting bodies growing in company with some of the largest carpophores collected. Although the striking color difference between rapidly developing fruiting bodies is the most conspicuous difference between *C. Kauffmanii* and *C. floccosus,* there is a characteristic difference in aspect which enables one to distinguish even the old worm-eaten faded carpophores of each species at a glance.

7. CANTHARELLUS FLOCCOSUS Schweinitz, Trans. Am. Phil. Soc. II. **4**: 153. 1832. f. TYPICUS (FIG. 7, center and left).

Cantharellus princeps Berkeley & Curtis, Ann. Mag. Nat. Hist. III. **4**: 293. 1859.

ILLUSTRATIONS: Peck, Mem. N. Y. State Mus. **3**: pl. 55, figs. 1–9; Marshall, N. Mushroom Book, pl. 45; Peck, Rep. N. Y. State Mus. **33**, pl. 1, figs. 18 & 20; Hard, Mushrooms, pl. 23, fig. 160, p. 201. 1908; White, Conn. State Geol. & Nat. Hist. Surv. Bull. No. 15, pl. 19; Bresadola, Iconographia Mycologica **26**, pl. 475.

Pileus 5–10 (15) cm. broad, 8–15 (20) cm. high, truncate when young, soon becoming hollowed in the center, the margin ascending but finally spreading and then the cap vase-shaped or trumpet-like, the interior (upper) surface innately scaly from the breaking up of the surface layer, the scales appressed toward the margin, more recurved in the tube and orange-yellow to reddish orange, the interspaces yellowish (scales "apricot orange" more or less); flesh moderately thick, thin in old caps, white or pallid, unchanging, fibrous, odor and taste not distinctive; lamellae foldlike and very frequently forked or anastomosing, on old caps occasionally almost poroid, decurrent almost to the base of the stipe in an irregular manner; stipe short and not sharply distinct from the

pileus, solid at first but becoming hollow as the cavity in the pileus deepens, whitish and unpolished, tapered more or less to the base which is usually deeply sunken in the humus.

Spores ochraceous in deposits, 12–15 × 6–7.5 μ, narrowly ellipsoid, slightly yellowish in KOH, ochraceous tawny in iodine, exospore slightly wrinkled; basidia 52–60 × 10–12 μ, clavate, hyaline in KOH, yellow in iodine; cystidia none seen; gill trama interwoven and not clearly distinct from that of the pileus, hyaline in KOH, yellow in iodine, no clamp connections present.

HABIT, HABITAT AND DISTRIBUTION: During the late summer and fall this is a common species in the higher mountains, and with the onset of the rainy season may be expected in most any conifer area from sea level to timberline. The carpophores may occur singly, gregariously or subcespitosely, often at higher elevations with two or three developing from a common stipe. It is one of the common fungi in the area and during moist warm weather attains its maximum size and brilliancy of coloration.

DISCUSSION: The fruiting bodies of this species are very persistent with the result that one frequently finds it in the faded condition and then an inexperienced collector might confuse it with *C. Kauffmanii.* It lacks the pungent odor of *C. Kauffmanii,* and this will often help to distinguish collections of carpophores which are past their prime. Fresh material which is still developing vigorously is readily identified by the orange to orange-red color of the scales. As found throughout the area concerned, there is considerable difference in stature to be noted on collections made in different seasons and at various elevations, but we have not separated out taxonomic units other than forms on the basis of whether the fructification becomes vase-like and the stipe deeply hollowed. The publication of this paper was withheld for a number of years in order to study these variations carefully. During the seasons of 1944 and 1946 extensive collecting was carried out along the crest of the Cascades between Mt. Hood and Mt. Jefferson. Many collections with solid stipes and somewhat truncate pilei were found along with some from relatively warm, wet areas which had deeply hollowed stipes. It appears to us that these differences can be satisfactorily explained by the difference in growth rate shown by carpophores from high elevations as compared with those from lower areas. In f. *typicus* the stipe is solid

at first but the cap expands fairly rapidly under average conditions so that the stipe soon becomes hollowed by the breaking up of the stipe tissue into soft scales which soon weather into a more or less floccose condition. The extreme in this type of development is found on fruiting bodies from the redwood forests along the coast of California. The other extreme is an alpine type often with several pilei arising from a common stipe as in *C. Bonarii,* but with larger caps, solid stipes, well formed fibrous and often very persistent scales (which usually are weakly tinted yellow-orange in color). Groups of these caps have been observed to change but little in a two-week period. Since high altitudes in this region do not favor either decay or insect damage to carpophores, those of this species very likely develop over a period of at least a month, particularly during cool dry years. Figures 7, 8 and 9 show three of these variants. For an illustration in color of f. *typicus* see Smith.[1] Since the extreme variants are fairly constant in the areas where the conditions producing them are constant, we have given them the ranking of formae and distinguish them as follows from f. *typicus.*

(a) C. FLOCCOSUS f. **rainieriensis** f. nov. (FIGS. 9 & 10). A typo differt: Pileus squamosus; stipes solidus vel sursum excavatus. Specimen typicum legit C. Frank Brockman, Mt. Rainier National Park, Wash.; in Mt. Rainier National Park Herbarium conservatum.

The scales of the pileus vary from the condition shown here to that found in f. *typicus.* The subalpine form is essentially a short-stiped slow growing form of heavy stature, and in which the stipe seldom becomes deeply hollowed. The configuration of the hymenium is more frequently like that of *C. Kauffmanii* than is that of any of the other forms. Although essentially alpine to subalpine in distribution, during cold dry years one can expect to find it at relatively low elevations and in such seasons it intergrades imperceptibly with f. *typicus.*

Cantharelli with solid stems which do not readily become hollow were first called to our attention by C. Frank Brockman in 1930 while he was naturalist at Mount Rainier National Park. His

[1] Mushrooms and other fleshy fungi in their natural habitats. Illustrated with stereo-kodachromes. Sawyer's Inc., Portland, Ore. In press.

FIG. 7. Left: *C. floccosus* f. *excavatus* (16 × 9 cm.). Right and Center: *C. floccosus* f. *typicus*.

original collection constitutes the type and is on deposit in the herbarium of the Park at Longmire, Washington. More recently the form was collected along the crest of the Cascades between Mount Hood and Mount Jefferson in Oregon by Smith and photographs obtained.

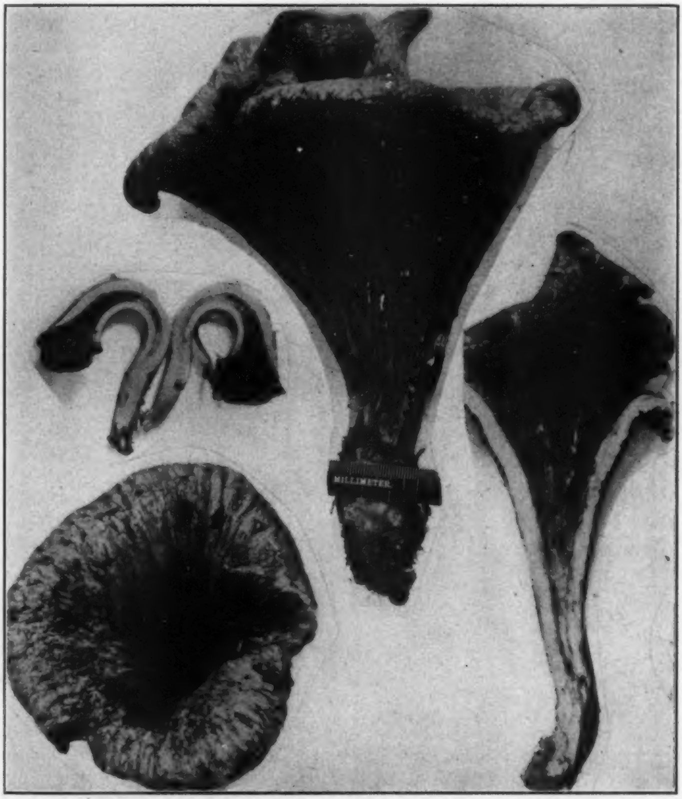

FIG. 8. *C. floccosus* f. *excavatus*. × ⅓.

(b) C. FLOCCOSUS f. **Wilsonii** f. nov. (FIG. 11). A typo differt: Stipes solidus, pileus squamosus, truncatus. Specimen typicum in Morse Coll. et in Herb. Univ. of Mich. conservatum; legit prope Wright's Lake, Sierra Nevada Mts., Calif., 6767 ft. elev. Oct., 1937.

FIG. 9. *C. floccosus* f. *rainieriensis.* ×1.

This form is paler than either f. *typicus* or f. *rainieriensis* but this is not so important as the solid stipe and nearly flat scaly pileus. Even when dried the stipe tissue (FIG. 11) does not collapse as in most other forms. Actually this form may represent a distinct species between *C. floccosus* and *C. Bonarii,* but so far we have seen only the one collection and it does not seem advisable to establish a species in this group without a detailed knowledge of all stages of development.

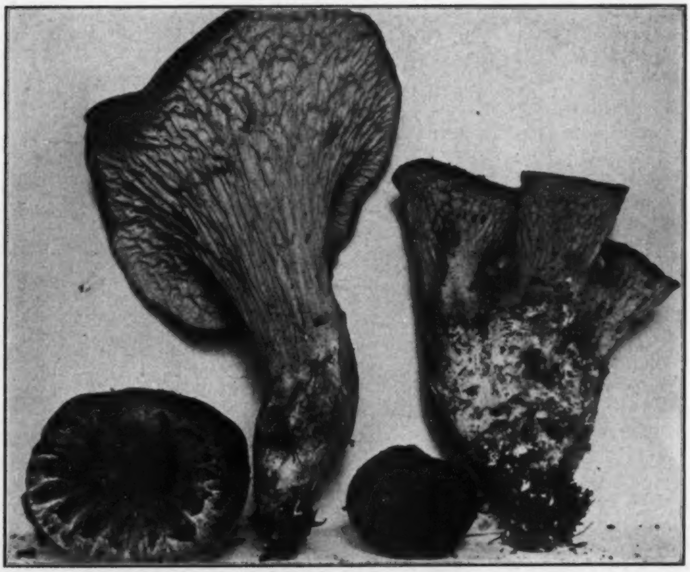

FIG. 10. *C. floccosus* f. *rainieriensis.* × ¾.

(c) C. FLOCCOSUS f. **excavatus** f. nov. (FIG. 7, left, and FIG. 8). A typo differt: Pileus profunde excavatus, floccosus; stipes deorsum solidus demum cavus. Specimen typicum in Morse Coll. conservatum; legit prope Eureka, Calif., 1930, Richardson.

This form is the largest of any in the species, and appears to be a robust, warm weather form characteristic of the redwood belt. As a rule the scales are not well developed, the cap being merely floccose to fibrillose. The colors are not as bright in

FIG. 11. *C. floccosus* f. *Wilsonii*.

most collections, being more yellow than red. The illustration of dried fruiting bodies (FIG. 8) is included to show the extreme difference between this form and f. *Wilsonii*.

8. CANTHARELLUS BONARII Morse, Mycologia 22: 219. 1930.

ILLUSTRATIONS: l.c. pls. 24 & 25.

Pileus 3–7 cm. broad, fleshy, margin involute at first, regular in young carpophores, spreading and undulating or lobed at maturity, soon depressed at the center, surface broken into thick floccose more or less erect scales which fill the central depression, scales orange at the tips blending to lemon yellow at the base and giving the entire cap an orange-yellow color, fading to near "pinkish buff" in drying; flesh white, firm, tapering to margin, relatively thin, odor none, taste not recorded; lamellae obtuse, very narrow, in the form of radially disposed subdistant decurrent folds or meruloid (subporoid from anastomoses or connecting veins), primary folds sometimes decurrent half the length of the stipe, color milk-white when fresh, sordid cremeous to brownish when dried; stipe 2–4 cm. long, 10–15 mm. thick, solid, glabrous, white, enlarged upward into the pileus, mostly fused with other stipes (up to 13 from a common base), the entire cluster 5–7 cm. tall, many undeveloped fruiting bodies sometimes present in the large clusters.

Spores slightly yellowish as revived in KOH and viewed in groups, individual spores hyaline, probably yellowish in deposits, 10–12 (14) $\times$ 5–6 μ, subellipsoid, smooth to slightly roughened (outer wall slightly wrinkled), apiculate, ochraceous tawny in iodine; basidia two- to six-spored, hyaline in KOH, 44–70 $\times$ 7–8 μ, narrowly clavate with flexuous pedicels; no pleurocystidia or cheilocystidia present; gill trama of interwoven hyaline hyphae without clamp connections; pileus trama hyaline in KOH, the hyphae similar to those of the gill trama.

HABIT, HABITAT AND DISTRIBUTION: Closely gregarious to cespitose, partly hidden in deep humus under pine and fir, Sierra Nevada Mountains, California. It usually fruits in the spring.

DISCUSSION: This species is clearly related to *C. floccosus* but distinct by the smaller size of the fruiting bodies and the clustered manner of growth. A very distinctive feature is the large number of undeveloped fruiting bodies in some of the clusters and the tendency for a number (up to 13 carpophores) to be borne on a common base (l.c. pl. 24). Although *C. floccosus* is a common

fungus northward in the mountains of this region and one we have studied in great detail, we have never observed forms which intergraded with *C. Bonarii*. In the reaction of the spores in iodine, lack of clamp connections on the hyphae of the flesh, and in the character of the outer spore wall, the two are very similar.

9. **Cantharellus Wilkinsae** Morse, sp. nov. (FIG. 12)

Fructificationes 4–11 cm. altae, 2–4 cm. latae; pileus anguste infundibuliformis, saepe 3–6 lobatus, siccus, fibrillosus demum squamosus, pallide alutaceus, durus; lamellae anguste plicosae, decurrentes, pallide luteo-alutaceae; stipes sursum expansus, brevis, durus, solidus, pallidus; sporae 14–18 × 6–7 μ, leves.

Fruiting body 4–11 cm. high, 2–4 cm. broad at apex, not distinctly differentiated into pileus and stipe, apex with a flaring margin causing carpophores to be narrowly trumpet-shaped at maturity, margin soon splitting into 3–6 lobes which become decurved or may remain uplifted, the upper surface (inner surface of the trumpet) dry and fibrillose, the outer layer of it soon splitting into scales which curve inward toward the tube as in *C. floccosus* and eventually become worn away, or surface merely fibrillose-roughened and gradually glabrescent, color more or less sordid cinnamon buff and unchanging when dried; flesh hard and almost woody in old carpophores, odor and taste not recorded; hymenium smooth to longitudinally wrinkled or in the form of gill-like folds near the recurved segments, hymenium extending downward about half the length of the trumpet, sordid cream color and drying to pale sordid alutaceous; stipe not distinct from remainder of fruiting body and usually represented by the narrowed portion of the carpophore below the hymenium, solid, hard, semi-woody when dried, whitish throughout, base covered by white mycelium causing debris and dirt to adhere to it, clusters arising from a mass of underground fungous tissue.

Spores subellipsoid to slightly inequilateral, 14–18 × 6–7 μ, smooth, hyaline in KOH, yellowish in iodine or pale tawny if groups are viewed, the wall minutely punctate as seen mounted in iodine; basidia 72–90 × 9–12 μ, four- to eight-spored, yellowish in KOH (hymenium yellowish orange); pleurocystidia and cheilocystidia not differentiated; gill trama and pileus trama similar, hyaline in KOH, of very compactly interwoven hyphae 5–12 μ broad, yellowish in iodine, thin-walled, no clamp connections present.

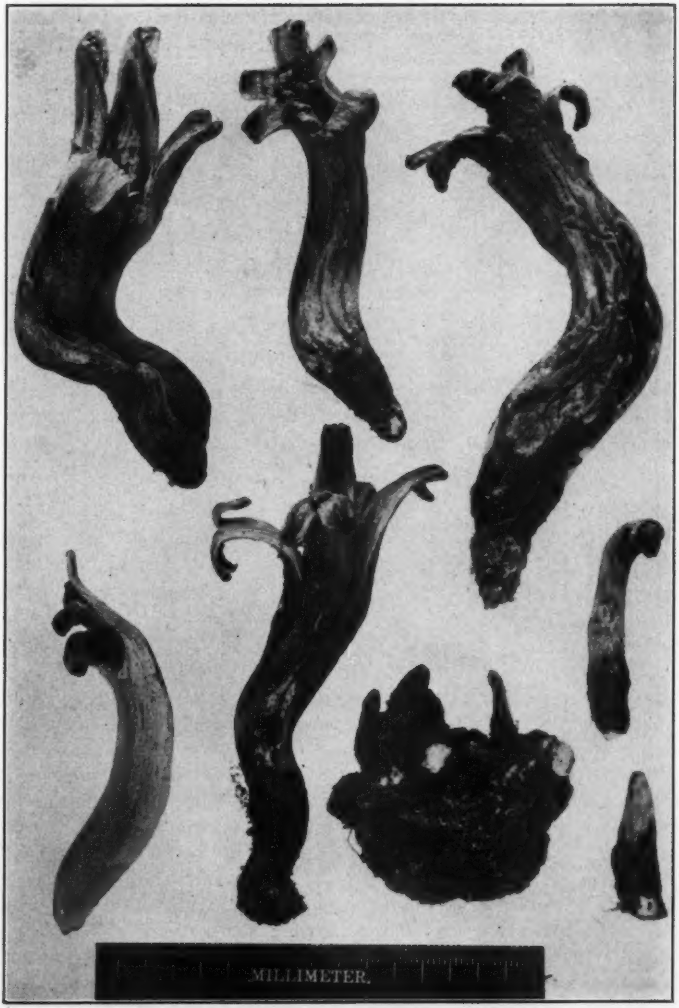

FIG. 12. *C. Wilkinsae.*

HABIT, HABITAT AND DISTRIBUTION: Clustered to gregarious in the vicinity of red fir, El Dorado National Forest, California, elev. about 6500 ft. The species is known only from the type collection. The fruiting bodies in some groups were so close to fir roots that they appeared to come from them but no real attachment was found.

DISCUSSION: This species is named in honor of Miss Edith Wilkins of Sacramento, California, who first discovered it near the Bryant Creek Tract in the El Dorado National Forest. Although the specimens were collected after a light fall of snow, it is obvious from the nature of the carpophores that they develop slowly and persist for a long time. Hence the species is hardly to be regarded as one of those which typically fruit late. The photograph illustrates all stages of development. In our estimation this fungus is properly classified in the same group as *C. floccosus* to which it is related by its microscopic characters, but represents a distinct species close to *C. Bonarii.* It is amply distinct from the latter by its larger spores, different colors and stature and much harder consistency.

10. CANTHARELLUS TUBAEFORMIS Fries, Syst. Myc. **1**: 319. 1821 (FIG. 13).

Pileus 1–3 (5) cm. broad, convex to plane or broadly depressed and with an arched incurved margin at first, margin finally spreading or uplifted and becoming crenate to variously lobed, occasionally somewhat funnel-shaped in age, usually not perforated in the disc at first but frequently becoming so in age, surface moist and more or less uneven, at times with radial ridges terminating in scabrous points, sometimes quite rough, sometimes practically glabrous, moist, color "Saccardo's umber" to "sepia" (dark sordid yellowish brown), at times more or less sordid ochraceous; flesh thin and membranous, fragile, yellowish to avellaneous, odor and taste not distinctive; lamellae decurrent, narrow and foldlike, subdistant, dichotomously forked, yellowish gray to "avellaneous" (grayish brown), often pale drab in age; stipe 3–6 cm. long, 3–7 mm. thick, stuffed but becoming hollow and flabby, subequal, often compressed or furrowed, glabrous, dark to pale ochraceous above, usually whitish at the base.

Spores white to creamy white in thick deposits, (8) 9–11 × 5.5–7 μ, ellipsoid to ovoid, smooth, not amyloid (pale ochraceous tawny

FIG. 13. *Cantharellus tubaeformis.* × 1.

in iodine); basidia 64–82 × 9–11 μ, clavate, flexuous toward the base, two- and four-spored, pale yellowish brown in iodine, content mostly of oil globules when revived in KOH; pleurocystidia and cheilocystidia none; gill trama interwoven, the hyphae 6–10 μ in diam. and hyaline in KOH, clamp connections present; pileus trama interwoven, of hyaline hyphae 6–12 μ in diam., the hyphae on the surface yellowish brown but otherwise not differentiated from the flesh proper, clamp connections abundant.

HABIT, HABITAT AND DISTRIBUTION: On wet soil, often along streams or near springs or in bogs under conifers. It varies from cespitose to gregarious. Its abundance and distribution in the area remain to be established. It has been collected along the East Fork, Salmon River, Mt. Hood, Oregon, at an elevation of about 3800 ft. (*S–19149*).

DISCUSSION: The nomenclature as well as the concept of this species is in a state of confusion. If one adheres strictly to the rules of nomenclature the name *C. tubaeformis* must be used by those who consider this and *C. infundibuliformis* to be synonyms. The carpophores of the various species in this group are so variable in color, so similar in aspect and in having the disc perforated in old specimens that we have been unable to arrive at satisfactory species concepts with them as a basis. In the American literature we find that *C. infundibuliformis* is said to have spores which are yellowish in a deposit whereas those of *C. tubaeformis* are white to creamy white. In the western United States we find collections which fall into these two categories, and we are recognizing the two species on that basis. It should be pointed out, however, that European authors are not in agreement on this point and one encounters the statement (see Rea, Brit. Basidiomycetes, p. 544) that both have white spores. We wish to emphasize that we are here drawing our concepts along the lines laid down by previous American workers because they seem to us to have hit upon the best diagnostic character—the one which clearly separates two taxonomic units at the species level in this complex. A third species, *C. lutescens,* has recently been studied from collections made near the Univ. of Michigan's Biological Station at Douglas Lake, Michigan. Its spore deposit is "ochraceous salmon" and its gills are not well formed. There are still other elements in this complex which need critical study.

11. CANTHARELLUS INFUNDIBULIFORMIS Fries, Epicr. Syst. Myc. p. 366. 1838.

Pileus 15–30 mm. broad, convex with a flattened or slightly depressed disc at first, soon deeply and sharply depressed and then becoming perforated, surface moist and uneven, with a yellow ground color on which are dispersed minute grayish streaks which may terminate in minute scales, color-effect about "Brussels' brown" in young caps, yellower in age; flesh thin, dull yellow, odor and taste not distinctive; lamellae pale yellow ("warm buff"), well formed and dichotomously forked, intervenose, narrow, decurrent, subdistant, edges obtuse; stipe 5–8 cm. long, 4–9 mm. thick, hollow, fleshy-fragile, glabrous, "light orange yellow" when young, paler and duller in age, sometimes a gray cast present near apex, base pale yellow.

Spores pale yellow in mass and yellowish when fresh material is mounted in water, bright yellow in iodine but fading to nearly hyaline on standing an hour, 9–11 × 7–8.4 μ; basidia up to eight-spored, yellowish orange in iodine in sections of dried material, paler after standing; no cystidia seen; subhymenium much branched, pale yellow in iodine; flesh of cap and gill trama compactly interwoven and sordid yellow to bister (thick sections) in iodine; cuticle not differentiated, clamp connections present on most hyphae of cap and lamellae.

HABIT, HABITAT AND DISTRIBUTION: Very common on conifer logs which have been reduced almost to humus. It may grow single, gregarious or cespitose, and fruits during the fall rainy season at low as well as high elevations.

DISCUSSION: As pointed out under *C. tubaeformis* these two differ primarily in the color of the spore deposit. No chemical differences have yet been found which seem to be worthy of mention. The iodine reaction of the spores is variable, being a bright yellow to almost pale ochraceous tawny in some collections, but if left standing in the medium they become paler to nearly hyaline. We do not insist that we have described here the *Cantharellus infundibuliformis* of European authors, but, as mentioned previously, seek to establish the existence of two distinct species in our *Cantharellus* flora. The name *C. infundibuliformis,* if the rules of nomenclature were strictly applied, would automatically become a synonym of *C. tubaeformis* (see Lange, Flora Agaricina Danica **5**: 85). This would appear to leave the yellow spored

species here treated as *C. infundibuliformis* without a name unless some little used name such as *C. sphaerosporus* Pk. were brought back into use. Here, however, we encounter the same difficulty which makes a decision on any name in the group difficult. The essential data are not given in the original description and cannot be obtained now from the type. In view of this confused situation we have decided to follow the concepts as they were given by Kauffman in his Agaricaceae of Michigan.

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NEW AND INTERESTING SPECIES OF CORDYCEPS [1]

E. B. Mains

(WITH 3 FIGURES)

In continuation of investigations concerning the genus *Cordyceps,* collections have been obtained from various sources which furnish additional information concerning the genus.

Cordyceps washingtonensis

In 1941 while located at Mount Baker, Washington, with A. H. Smith, the writer obtained several collections of a *Cordyceps* on lepidopterous larvae along the trails near Baker Lake. At the time, it was thought that a form of *Cordyceps militaris* (Fr.) Link had been obtained. The clavae resembled those of that species, differing principally in color which was sulphur yellow (R) [2] to Pinard yellow (R) instead of the orange or orange yellow of *C. militaris.* However, a study of the specimens has resulted in finding that they differ in several important microscopic details from that species. Apparently they belong to a new species which is described as follows.

Cordyceps washingtonensis sp. nov. (FIG. 1). Clavis fusoideo-cylindraceis vel anguste clavatis, 1.5–3.0 cm. longis, 2–6 mm. latis, sursum sulphureis, stipitibus albidis; peritheciis immersis in molle stromate denique prominentibus, sulphureis vel flavis, ovoideis, 480–644 × 252–386 μ; ascis cylindraceo-clavatis, deorsum attenuatis, 300–418 × 3–3.5 μ; ascosporis anguste cylindraceo-clavatis, 80–110 × 1–1.5 μ, non frangentibus.

Ex pupis lepidopterarum. Specimen typicum, Baker Lake, Washington, 1 Sept., 1941, E. B. Mains (*6185*), in Herb. Univ. Mich. conservatum.

Clavae fusoid-cylindric to narrowly clavate, 1.5–3.0 cm. long, 2–6 mm. wide, in the upper part sulphur yellow (R) when young becoming Pinard yellow (R) when mature, the stipes whitish; perithecia at first embedded in a soft whitish stroma, finally pro-

[1] Paper from the Department of Botany and the Herbarium of the University of Michigan.

[2] Colors designated (R) are from Ridgway, Color standards and color nomenclature.

jecting up to one-half of their length, at first sulphur yellow (R) becoming Pinard yellow (R) upon maturity, ovoid, 480–644 × 252–386 μ; asci cylindric-clavate, attenuated below 300–418 × 3–

FIG. 1. Clava of *Cordyceps washingtonensis*. × 3.

3.5 μ; ascospores narrowly cylindric-clavate, narrowed below, 80–110 × 1–1.5 μ, not breaking into segments.

From buried lepidopterous larvae, Baker Lake, Washington, September 1, 1941, E. B. Mains (*6185*, **type**); Sept. 2, 1941.

E. B. Mains (*6204*); Sept. 5, 1941, E. B. Mains and A. H. Smith (*6232*).

The description with the exception of the microscopic characters was made from the freshly collected specimens. The dried specimens are 1.1–2.5 cm. long and 1–4 mm. wide. They are sordid white except for the perithecia which are honey yellow. The soft white stroma in which the perithecia are embedded dries down to a very thin subiculum covering only the lower portions of the perithecia and leaving them almost superficial. In this respect it resembles *C. militaris*. Although it is similar to that species in general morphology it differs not only in color but in the types of asci and ascospores. *C. militaris* has cylindric asci and filiform ascospores which break up into one-celled fragments at maturity.

CORDYCEPS OLIVASCENS

Among the collections of fungi received from Dr. R. P. Burke of Montgomery, Alabama, in 1942, was a very interesting specimen of *Cordyceps*. It consisted of a single clava 4 cm. long with a cylindric head 1.5 cm. long and 2.5 mm. thick and the remains of an insect of which only chitinous fragments were left. The dried specimen is citrine-drab (R). Dr. Burke's notes give the color of the fresh specimen as olive buff (R) to deep olive buff (R). This appears to differ from other olivaceous species and the name *Cordyceps olivascens* is proposed for it.

Cordyceps olivascens sp. nov. Clava 4 cm. longa, olivacea, capitulo cylindraceo, 1.5 cm. longo, 2.5 mm. lato, stipite 1–2 mm. lato; peritheciis recte immersis, conoideis, 900–1200 × 240–374 μ; ascis anguste cylindraceis, 480–600 × 4–6 μ; ascosporis filiformibus, multiseptatis, frangentibus, articulis oblongis, 3–4 × 1 μ.

Ex insecto. Specimen typicum, Fusihatchie, Alabama, R. P. Burke, 21 Junii, 1942, in Herb. Univ. Mich. conservatum.

Clava 4 cm. long, the fertile portion cylindric, 1.5 cm. long, 2.5 mm. wide, citrine drab (R), punctate due to the slightly projecting ostioles of the perithecia, the stipe 1 mm. thick above, 2 mm. below, dark olive (R); perithecia conoid, 900–1200 × 240–374 μ, embedded with the longitudinal axis at right angles to the surface of the head; asci narrowly cylindric, 480–600 × 4–6 μ; ascospores filiform, multiseptate, soon breaking into oblong fragments 3–4 × 1 μ.

Associated with the remains of an unidentified insect in rotten wood humus under a log, Fusihatchie, Alabama, June 2, 1942, R. P. Burke (**type**).

The above description was made from the dried specimen. According to the notes of Dr. Burke the clava when fresh was lighter in color, olive buff (R) to deep olive buff (R). The stipe was almost white at the base and it was glabrous, finely striate and tubular. The insect was badly disintegrated, only fragments of the body wall, head and legs remaining.

There are a few species of greenish or olivaceous *Cordyceps* Of these, *C. olivacea* Rick, *C. olivaceo-virescens* P. Henn. and *C joaquiensis* P. Henn. are nearest to *C. olivascens*. *C. joaquiensis* was based by P. Hennings (**3**) upon a collection made by E. Ule in Brazil. It is described as having single or cespitose, fusco-olivaceous clavae, 6–8 cm. long. The asci are given as 150–200 × 4–4.5 μ. It is therefore a much larger species than *C. olivascens* and has smaller asci.

Cordyceps olivaceo-virescens was described by P. Hennings (**2**) from a collection from Brazil as clavate, furcate, olivaceo-virescent, with a fusoid or cylindric head 1.5–2 cm. long and 2–3 mm. thick and a stipe 3–3.5 cm. long and 1.5–2 mm. thick. The perithecia are given as semi-immersed, ovoid and 200 × 150 μ and the asci as cylindric, 120–160 × 3.5–4 μ, with filiform, multiseptate ascospores, 0.3–0.5 μ thick. Petch (**7**) casts some doubt on the color stating that it is "due, at least in part, to a covering of conidia of *Aspergillus* or *Penicillium*." He states that the perithecia are immersed and are oblique as in *C. brasiliensis* for which he considers it synonymous.

Cordyceps brasiliensis was described by P. Hennings (**1**) from a collection from Brazil as 5 cm. long, 2 mm. thick, pallid and furcate at the apex. The perithecia are given as oblong, 0.7–0.8 mm., free or adnexed and the asci as 220–290 μ long and 4–5 μ wide. Petch (**7**) states the the perithecia of the type are immersed and oblique. Since it was preserved in alcohol as an exhibition specimen he was not able to obtain additional details. The pallid color was doubtless due to its preservation in alcohol and the original color therefore is uncertain. It seems question-

able therefore that the name *C. brasiliensis* should be applied to *C. olivaceo-virescens.*

C. olivaceo-virescens has been reported from China by Teng (**12**). He describes the clavae as olivaceous green, 4–6 cm. long, solitary to gregarious and arising from a dense greenish mycelium which almost entirely covered the host, a small Coleoptera. The stipe is simple or branched, 2.5–4.5 cm. long and 1.5 mm. thick, and the head cylindric, 15 × 2–3 mm. The perithecia are obliquely immersed, ovate, 550–600 × 300 μ and the asci 450 × 4 μ. As Teng notes, the size of the perithecia and asci differs considerably from those given by Hennings. He points out that if Hennings' measurements were obtained from transverse sections of the head, they would be erroneous.

Cordyceps olivacea Rick was proposed by C. G. Lloyd (**4**) for a collection sent by J. Rick from Brazil. He published two illustrations and stated that it resembled *C. militaris* except that it was smoother and differed in color which was olive when fresh (**4**, p. 1308). He suggested that it might be the same as *C. olivaceo-virescens.* Petch (**7**) considers it a synonym of *C. brasiliensis* along with *C. olivaceo-virescens.* Through the kindness of J. A. Stevenson the specimen of *C. olivacea* in the Lloyd Herbarium at Washington was loaned for study. This consists of one clava which now shows no trace of green. The information derived from this specimen follows:

Cordyceps olivacea Rick. Clava club-shaped, 5 cm. long, with the fertile portion 2 cm. long, 4 mm. thick, dull yellowish brown, punctate due to darker colored ostioles; stipe 3 cm. long, 1.5 mm. thick; perithecia entirely embedded, oblique to the surface of the clava, flask-shaped, 600–780 × 300–360 μ; asci cylindric, 450–480 × 4–5 μ, the ascospores nearly as long as the asci, 1 μ thick, multiseptate, with the septa 5–15 μ apart. Lloyd Herb. *35621.*

Cordyceps olivacea is apparently the same as *C. olivaceo-virescens.* The oblique perithecia effectively separate the species from *C. olivascens.*

Teng (**12**) has reported a collection from China on a coleopterous insect as *C. olivacea.* He describes the clavae as solitary, 4–5 cm. long, 1.5–2 mm. thick, olivaceous when fresh, umber when dry and the perithecia as immersed, ovate, 320–380 × 150–160 μ

with asci 220–250 × 8 μ. The orientation of the perithecia is not described. Since in the immediately preceding discussion of *C. olivaceo-virescens* great emphasis is placed on oblique perithecia, it is probably safe to assume that the perithecia in the specimens reported as *C. olivacea* are arranged at right angles to the surface of the clava. It therefore is not *C. olivacea*. It differs from *C. olivascens* in the much smaller asci. It may be *C. joaquiensis*. The latter is illustrated as very cespitose but is described as cespitose or solitary. The asci are described as much narrower, 4 μ.

CORDYCEPS CURCULIONUM (TUL.) SACC.

Cordyceps curculionum was described by Robin (**9**) under the name *Sphaeria* (*Cordyceps*) *entomorhiza* Dickson. Tulasne (**13**) pointed out that it was not that species and proposed the name *Torrubia curculionum* for it. Robin described and illustrated a *Cordyceps* having long, slender, simple or furcate, bicolored clavae with ovoid heads. The heads and upper part of the stipes are light yellowish and the lower portions of the stipes a brownish black. According to Saccardo (**10**) the heads of the clavae are clavate to ovoid, 2–2.5 × 1.5–2 mm., and flavo-griseus and the stipes 18 mm. long, 0.25–0.5 mm. thick, whitish above and brownish below. The asci are described as cylindric, 300–350 × 4–6 μ, and the ascospores as filiform breaking into elongated ovoid segments, 8–9 × 1.5 μ.

Spegazzini (**11**) described a similar fungus from Brazil under the name *Cordyceps Puiggarii*.[3] The stipes are described as 3–6 cm. long, 0.5 mm. thick, fusco-ater below and carneo-luteus above. The heads are elliptic-sublimoniform, 2–3 × 1.5–1.75 mm., very smooth and carneo-luteus. The perithecia are 800 × 100 μ, entirely embedded and arranged horizontally. The asci are cylindric, 250 × 6–8 μ, and the filiform ascospores break into segments 5–10 × 1 μ. Petch (**8**) has stated that this is *C. curculionum*.

In a specimen from British Honduras reported by the writer (**5**) as *C. curculionum* the perithecia have been found to be oblique. They are narrowly conoid, 780–1020 × 264–300 μ, entirely embedded, overlapping upward with their longitudinal axes ob-

[3] As pointed out by Petch (**8**) the name *C. Puiggarii* was previously used by Spegazzini, for another species (probably *C. sphecocephala*).

lique to the surface of the head. This arrangement is illustrated by Robin (**9**) in the original description of *C. curculionum*. If the Brazilian collection has horizontal perithecia as described by Spegazzini it would belong in another species without a valid name. However the measurements given by Spegazzini for the perithecia in relation to those for the head would make a horizontal arrangement appear doubtful and the identity of the collections of Puiggari questionable.

CORDYCEPS SALEBROSA

In 1941 a specimen of a *Cordyceps* with bicolored clavae on a beetle was received from G. W. Martin. This differs from the preceding in several important aspects and is described as a new species as follows.

Cordyceps salebrosa sp. nov. (FIG. 2). Clavis 3.5–4.0 cm. longis, capitulis oblato-globosis, 1.0 mm. crassis, 1.5 mm. latis, pallide cremeis (lectis rubris), super salebrosis, stipitibus filiformibus, 0.2–0.3 mm. crassis, deorsum fusco-atris, sursum concoloribus capitulis; peritheciis immersis, anguste conoideis, 840–1200 × 240–300 μ; ascis anguste cylindraceis, 600–660 × 6 μ; ascosporis filiformibus, in articulis, 6–10 × 1–1.5 μ, frangentibus.

Ex coleoptero. Specimen typicum, Barro Colorado Island, Panama Canal Zone, 24 Julii, 1935, L. Hare in Herb. Univ. Mich. conservatum.

Clavae 3.5–4.0 cm. long, emerging between the thorax and abdomen of a beetle, capitate, the heads oblate-globoid, 1 mm. thick, 1.5 mm. wide, light cream color after being preserved in alcohol, reported as red when fresh, very irregular on the upper surface, the stipes filiform, 0.2–0.3 mm. thick, brownish-black below, concolorous with the heads in the upper 3–4 mm.; perithecia narrowly conoid, 840–1200 × 240–300 μ, vertical, completely imbedded in the head; asci narrowly cylindric, 600–660 × 6 μ; ascospores filiform, breaking into segments 6–10 × 1–1.5 μ.

From imago of a beetle (Elateridae), Barro Colorado Island, Panama Canal Zone, July 24, 1935, L. Hare (**type**).

The specimen was preserved in alcohol and the heads and short portions of the stipes immediately below the heads are now a light cream color and the remainders of the stipes are brownish black. According to the collector the upper portions of the clavae were red when fresh. The beetle was apparently attached to the substratum by a dense mat of brownish mycelium.

The flattened heads resemble those of *Cordyceps dipterigena* Berk. & Br. on flies. The upper surfaces of the heads are more irregular. Apparently this is due to the upper layer of the stroma

FIG. 2. Head of the clava of *Cordyceps salebrosa* showing flattened shape and irregular surface. × 40.

conforming to the upper portions of the perithecia. The bicolored clavae and the flattened irregular heads taken together distinguish this species from others previously recorded on beetles.

Cordyceps venezuelensis sp. nov. (FIG. 3, A & B)

Clavis numerosis, gracilibus, contortis, simplicibus vel irregulariter furcatis, 2 cm. longis, 0.8 mm. crassis, pallide luteo-brunneis; peritheciis superficialibus, liberis, dispersis et irregulariter confertis, ovoideis, 288–324 × 182–

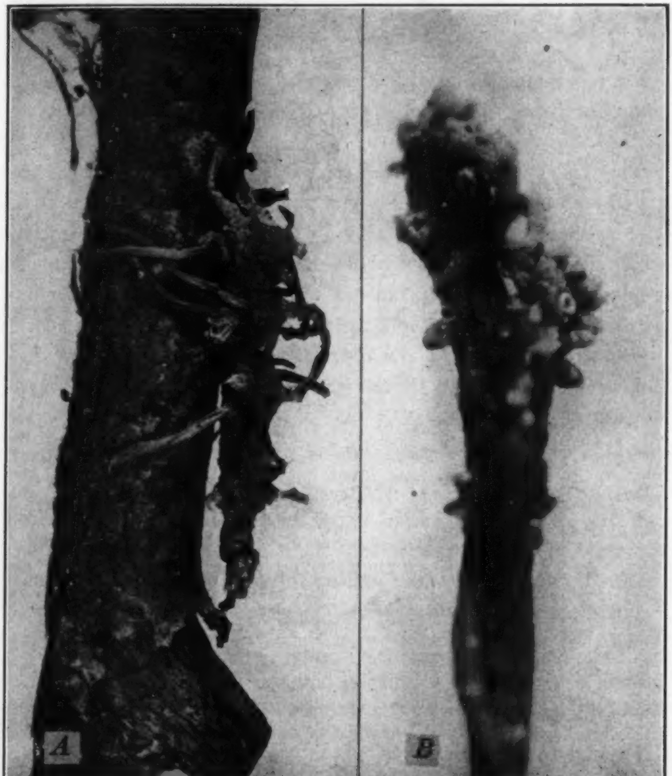

FIG. 3. A. Lepidopterous larva showing clavae of *Cordyceps venezuelensis* arising from various portions of the body. Approximately × 2.5.

B. Upper portion of a clava showing the superficial, irregularly distributed perithecia. × 15.

204 μ; ascis fusoideo-cylindraceis, 138–204 × 4–5 μ; ascosporis filiformibus, 108–130 × 1.5 μ, multiseptatis.

Ex larva lepidopteri. Specimen typicum, Yaracuy, Venezuela, 6 Jan., 1938, Carlos E. Chardon (*2557*), in Herb. Dept. Plant Path., Cornell Univ. conservatum.

Clavae numerous from all parts of the larva, very irregular, simple or irregularly furcate above, up to 2 cm. long, 0.8 mm. thick, twisted, light yellowish brown; perithecia ovoid, superficial, free, scattered and irregularly crowded in groups, with portions of the clavae without perithecia, 288–324 × 182–204 μ; asci fusoid-cylindric, 138–204 × 4–5 μ; ascospores filiform, 108–130 × 1.5 μ, not breaking into fragments.

On lepidopterous larva in dense forest, Central Lucinda, Yaracuy, Carababo, Venezuela, Jan. 6, 1938, Carlos E. Chardon (*2557*).

This collection was received from H. H. Whetzel who was engaged with Carlos E. Chardon in a study of the fungi of Venezuela. The species varies considerably in its development. The clavae which arise from all parts of the insect (FIG. 3A) are simple or produce short irregular branches near the apex. The superficial perithecia are very unevenly distributed (FIG. 3B). This species appears to be close to *Cordyceps flavo-brunnescens* P. Henn. It differs in having more slender clavae and broader asci and ascospores.

CORDYCEPS MYRMECOPHILA CES.

Cordyceps myrmecophila has previously (**6**) been reported from the state of Washington. Two additional collections have been received, one from Oregon and the other from British Columbia. From these the following description has been prepared.

Clavae 8–25 (40) mm. long, capitate, the heads ovoid, 2–4 × 1–2 mm., ochraceous to ochraceous salmon (R), irregularly slightly longitudinally ridged, the stipes slender, 0.3–1.0 mm. thick, concolorous with the head or somewhat lighter, sometimes whitish below; perithecia narrowly ovoid,[4] 600–1020 × 192–300 μ, entirely embedded except for the slightly projecting ostioles, extending upward and oblique to the surface of the head; asci narrowly cylindric, 480–720 × 4–6 μ; ascospores hyaline, filiform, breaking into one-celled fragments, 8–10 × 1.5 μ.

On ants, Vancouver, British Columbia, April 16, 1941, J. F. Davidson; Bruin Run Creek, Mount Hood, Oregon, September 24, 1944, Wm. B. Gruber and A. H. Smith (*19033*).

[4] The perithecia were previously (**6**) erroneously described as obovoid.

The Oregon specimens have the longest stipes, due apparently to the depth to which the infected ants were buried in the soil. This also resulted in a much lighter color for the lower portion of the stipes. When fresh the heads are almost smooth becoming slightly irregularly ridged due to the greater shrinkage of the stromata between the oblique upwardly projecting perithecia.

Mr. Davidson found the species was very prevalent on ants in one locality at Vancouver in 1941. When he revisited the locality in 1944, although less numerous, the fungus was still fairly abundant.

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THE IDENTITY OF "METARRHIZIUM GLUTINOSUM" *

W. Lawrence White and Mary H. Downing

(with 2 figures)

Shortly after the beginning of the war, members of the Division of Cotton and Other Fiber Crops and Diseases, United States Department of Agriculture, in connection with work on the preservation of cotton fabrics, obtained from stored baled cotton in Washington, D. C. (**4**) an isolate (*1334.2*) representing a then unknown species of fungus, and a little later (**2**, **4**) two additional isolates, *1334.1* and *1334.3,* representing the same species, from Maryland soils. Work there, based on *1334.1* (**3**) and perhaps to a lesser extent (**2**, **4**) on *1334.2* and *1334.3,* soon demonstrated the species to be highly adapted to use in laboratory work pertaining to the microbiological decomposition of cotton fabrics, and later (**4**) *1334.2* was made the type of a new species.

After the microbiological decomposition of military fabrics in the tropics became recognized as a serious problem and a program for the development of preventive measures got under way, these cultures were widely distributed to governmental, commercial, and university laboratories in this country and throughout the British Empire. Toward the end of the war *"Metarrhizium glutinosum,"* as the species was called in deterioration work, was used perhaps more than any other, not excluding the previously universally employed *Chaetomium globosum,* in the assessment of preservative treatments of fabrics. The species is now used in the Biological Laboratories of the Philadelphia Quartermaster Depot in research on the chemical nature of the fungus breakdown of cellulose, and is used elsewhere wherever similar work or assessment testing is being conducted. Its mineral nutrition, temperature, and pH relationship to cellulolytic activity were investigated recently in a Quartermaster sponsored project at the Pennsylvania State College but the results are not as yet published.

* Presented at the Meeting of the American Association for the Advancement of Science, Boston, December, 1946.

Finally the species was introduced to the field of antibiotics when British workers (1) extracted from filtrates of a culture obtained from the U.S.D.A. an antifungal substance which they named glutinosin. Extracted along with the glutinosin but remaining in the mother liquors after crystallization of the glutinosin was another biologically active material causing a dermatitis of humans similar to that caused by poison ivy, thus necessitating the wearing of barrier creams in working with culture extracts of the mold.

Though the culture first introduced to the literature was *1334.1*, it appears that *1334.2* was the one that was most generally and widely distributed, and on which most, if not all, later work in the various laboratories was based.

In view of the foregoing interest and activity, this note is presented for the purpose of calling attention to the need for further inquiry into the true identity and relationship of what is now masquerading under the name *Metarrhizium glutinosum*. The name is unfortunate from both the scientific and economic points of view, viz.: (1) it places the species as next of kin to an insect-inhabiting form to which presumably it bears no close relationship in biological activity, thus misleading workers interested in cellulolytic activity or antibiotics or other fields of endeavor, who may for obvious reasons wish to survey related forms; (2) it breaks the continuity of the literature, submerges earlier records, and stands in the way of co-ordination of effort among the various interested laboratories and individuals; and (3) the addition of new names to the literature for organisms with long prior records, and which ought to be identified, is a practice which must be more vigorously condemned in the future than it has been in the past.

What, then, is *"Metarrhizium glutinosum"* and where are to be found other species which are phylogenetically related and which might exhibit more or less similar cellulolytic, antibiotic, and other biological activity?

THE GENUS MYROTHECIUM

There is a genus *Myrothecium* which has been known—in a sketchy way, to be sure—for some 150 years. During this period it has accumulated about a dozen species. All are very inade-

quately known, but the best known and evidently the most common are what have been correctly termed by Preston (**5**), working in England, the three basic species: *M. inundatum* Tode ex Fr., *M. roridum* Tode ex Fr. and *M. verrucaria* (Alb. & Schw.) Ditm. ex Fr.

Dr. John Stevenson has kindly loaned the writers the *Myrothecium* folder from the general collection of the Pathology and Mycology Collections of the United States Department of Agriculture. It contains thirty-five packets, representing, according to the labeling, six species, of which twelve are said to be *M. roridum* and eleven *M. verrucaria.* Examination of the lot indicates the existence of considerable confusion in the nomenclature but that the collections do actually represent a group of at least six reasonably closely allied species. A re-sorting of these places fifteen specimens under *M. roridum* and four in *M. verrucaria.* A more complete treatment will have to await the appearance of a monograph of the genus, which is badly needed.

The attention of Preston (**5**) was drawn to the systematics of the genus in an attempt to identify a form actively pathogenic to the cultivated violet, *Viola tricolor,* in England. This he established as *M. roridum,* which was isolated also from tomato and snapdragon plants in England and from several species of plants in Sierra Leone, and which showed some indication of pathogenicity to several species. He recorded *M. verrucaria* from Southern Rhodesia, Cyprus, and the Sudan. The Cyprus culture was from living potato haulms and was believed to have been pathogenic. An isolate (made in 1943) from an old canvas shoe (possibly American) was said to be the first record of the species in the British Isles. It may be noted at this point that in North America *M. roridum* was reported (**6**) to have been the cause of a crown canker of snapdragons in a commercial greenhouse in Texas in 1933–34, killing 90 per cent of the plants, and that more recently it has received attention as a pathogen of greenhouse snapdragons in Colorado (**8**).

Preston appears to have established the identity of the "three basic species," previously mentioned, about as adequately as will ever be possible. The one weakness in his treatment of *M. verrucaria* is that the type, if still in existence, was not available for

his examination. Nevertheless, since his coverage of historical material and literature is good, his work is acceptable, and the species may be considered as adequately established in accordance with his treatment.

A comparison of the USDA *1334.2* culture with Preston's description and illustrations of *Myrothecium verrucaria* disclosed no detectable differences. The conclusion that the American cultures, under the name *Metarrhizium glutinosum,* were conspecific with the British cultures of *Myrothecium verrucaria* was confirmed by further comparisons as indicated on the following pages. Therefore, *Metarrhizium glutinosum* Pope should be referred to synonymy under *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr.

MORPHOLOGICAL COMPARISON OF BRITISH AND AMERICAN CULTURES

One of the cultures cited by Preston (**5**, p. 168) was requested and obtained by the writers from Mr. George Smith of the London School of Hygiene and Tropical Medicine, who had originally furnished it to Preston after it had been isolated from a canvas shoe in England. The culture when received at Philadelphia was given the number PQMD *185*. A detailed comparison of its microscopic characters with those of USDA *1334.2* revealed no differences whatsoever. When the two numbers were grown in parallel series on plates of potato dextrose agar (FIG. 1A, B) there likewise were no differences. A culture of *Myrothecium roridum* (PQMD *188*), furnished by Dr. Stevenson after it had been isolated from tomato fruits intercepted at the Mexican-United States border by quarantine inspectors of the U.S.D.A., was grown in the same series (FIG. 1C). Its growth pattern, as indicated in the photograph, was only slightly different. This difference is of no significance in the separation of the two species since both have often been observed to sporulate in definite and sharply delimited concentric zones. The two species are very closely related as evidenced not only by their morphology but also by their occurrence, distribution, and biological activity. The best, and perhaps the only, distinguishing character is the spores, which in *M. verrucaria* are more or less ovoid with a peculiar and characteristic outline,

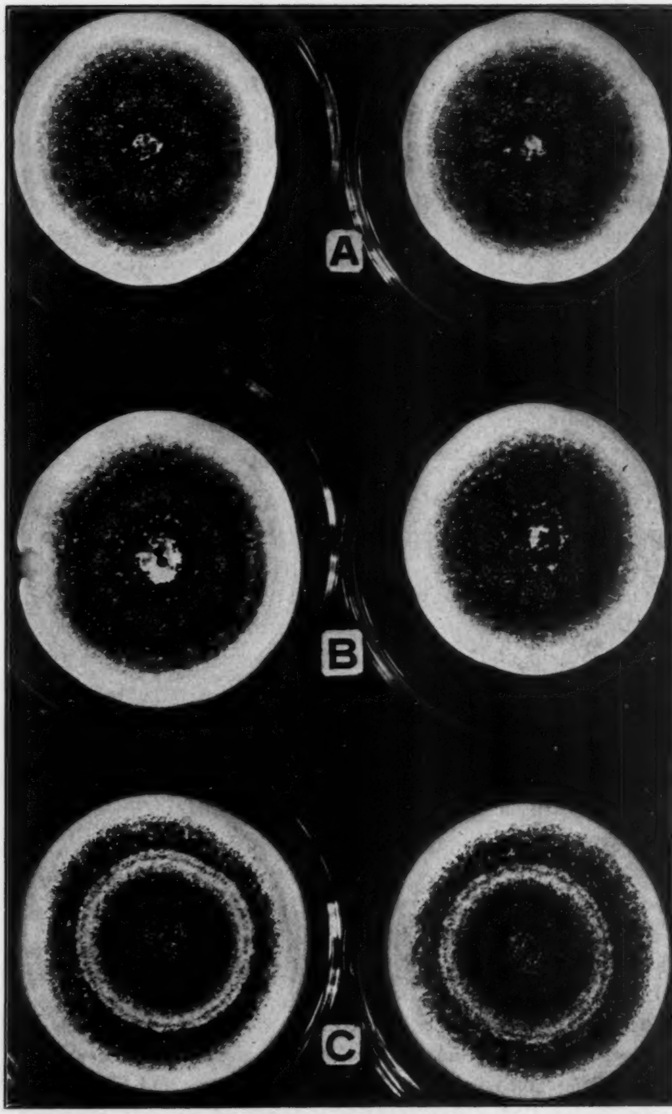

FIG. 1. *Myrothecium verrucaria* (A, B) and *M. roridum* (C) on potato dextrose agar.

whereas in *M. roridum* they are narrow and cylindric. The microscopic characters of *M. verrucaria* were adequately described and illustrated by both Preston and Pope, and of *M. roridum* by Preston.

More recently the remaining USDA cultures of "*Metarrhizium glutinosum*," *1334.1* and *1334.3*, were supplied by Dr. Paul Marsh and the additional cultures cited by Preston were obtained from him. They exhibit no noteworthy variation and all represent the same species. Preston's cultures of *Myrothecium roridum* were also made available.

CELLULOLYTIC ACTIVITY IN PURE CULTURE ASSAY

The cellulolytic activity of Cultures PQMD *185* and USDA *1334.2* representing *Myrothecium verrucaria* and PQMD *188* representing *Myrothecium roridum* was compared as follows:

Strips of 3.3 oz., relatively size-free, bleached cotton sheeting were raveled to a width of exactly 1 inch, cut to six-inch lengths and placed 1 each in 150 × 25 mm. test tubes each containing 25 ml. Greathouse Formula A mineral salts solution (**3**) with lower half of strip submerged, and autoclaved 20 min. at 15 lbs. pressure. Inoculum was prepared as follows: Each of the three organisms was grown on 2 per cent potato dextrose agar slants for 17 days. Formula A solution was placed in the tubes, the spores gently dislodged by agitation with the tip of a pipette, and the suspensions emptied into flasks containing glass beads and shaken. The spore counts were adjusted to approximately 22,000,000 per ml. for each of the three cultures to be tested. Two ml. of the resulting suspension was pipetted evenly over the exposed portion of each strip. Incubation was in a room set at approximately 85° F. and 80 per cent relative humidity. At harvest the strips were removed from the tubes, washed in 95 per cent alcohol, rinsed in tap water, dried in the laboratory atmosphere, then conditioned 24 hours in an atmosphere containing 65 per cent relative humidity, temperature 75° F., and broken on the motor driven Scott tensile strength tester with a three inch space between the jaws. Each figure in the following table represents pounds breaking strength retained, based on an average of ten replicates. The decline in

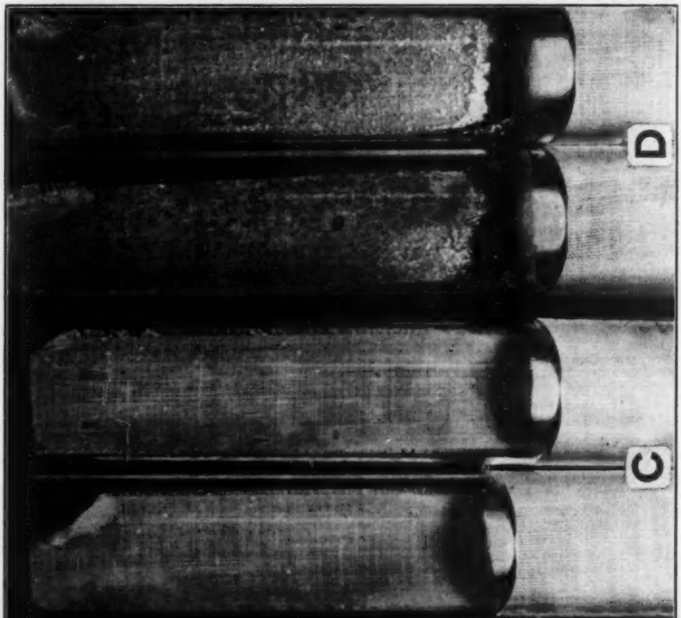

FIG. 2.

TABLE I

CELLULOLYTIC ACTIVITY OF MYROTHECIUM VERRUCARIA AND M. RORIDUM

Organism	Lbs. tensile strength retained		
	40 hours	64 hours	90 hours
PQMD *185* *M. verrucaria*	28.0	11.4	3.0
PQMD *188* *M. roridum*	40.7	30.5	14.8
USDA *1334.2* *M. verrucaria*	28.9	13.5	3.1
Uninoculated control	40.6	40.2	41.2

tensile strength of the cloth is taken as a measure of the cellulolytic activity of the organisms.

It will be noted from the table that under the conditions of the test the capacity of the two strains of *M. verrucaria* to break down cellulose was approximately equal whereas that of the single strain of *M. roridum* was somewhat weaker. The appearance of the three cultures on the fabric test strips after ninety hours incubation is shown (FIG. 2). Here again little or no difference is noted between the two strains of *M. verrucaria* (FIG. 2B, D). Mycelial development and sporulation is apparent over all of the exposed portions of the strips but is particularly heavy at and just above the surface of the liquid. In the case of *M. roridum* (FIG. 2C) visual growth was comparatively light and confined mostly to a rather scanty mycelial development on and just above the surface of the liquid, with little or no sporulation. In both strains of *M. verrucaria,* the cloth became intensely yellow just above the surface of the liquid and more faintly so progressively further up. In *M. roridum* a similar pigmentation was present but much less intense. As is typical of fungi tested by this method, cellulolytic action is greatest immediately above the surface of the liquid.

After the remaining cultures cited by Preston under *M. verrucaria* and *M. roridum* were received, these were also subjected to cellulolytic assay. The latter set of tests, although much less exact than the first, was sufficient to indicate that strong cellulolytic

action in pure culture is a characteristic common to both species. Of approximately one hundred species of fungi surveyed in this laboratory during the past two years, none has been found to exhibit stronger action. A few approach it, but the vast majority act more slowly, and of course many are wholly non-cellulolytic.

OCCURRENCE ON FABRICS AND PLANT MATERIALS IN THE FIELD

A preliminary examination of herbarium material from the U. S. Department of Agriculture, and to a lesser extent from other sources, together with a review of literature records, a few of which were cited on previous pages, indicates that both *M. verrucaria* and *M. roridum* are fairly common and of widespread occurrence in both North America and Europe and in the tropics. *M. verrucaria* appears to be the less common of the two species. Most of the specimens from the U.S.D.A. are on plant debris. However, some furnish additional evidence that both, but especially *M. roridum,* are capable of invading the living tissues of a wide range of herbaceous economic plants. One, for example, taken in Virginia in 1937 shows numerous sporodochia of *M. verrucaria* on a large necrotic area on a tomato fruit. The sporodochia of *M. roridum* are abundant in concentric rings of leaf spots of *Hibiscus* sp. from Honduras, and of *Citrus maxima* from Puerto Rico, presenting unmistakable evidence that *M. roridum* is the causal agent of the disease.

Among several thousand cultures at hand, made from deteriorated cotton fabrics and related military and industrial materials, mostly from tropical exposures, these two species appear only a few times. In a few instances *M. roridum* has been found sporulating on the fabric, but in no case has *M. verrucaria* been seen on the several hundred molded samples which have been thoroughly examined. As pointed out recently (7) by the senior author, *M. verrucaria,* despite its strong cellulolytic activity in pure culture laboratory tests and its special adaptability to laboratory experimental work, appears to be of little significance as a destroyer of cotton fabrics in the field.

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EXPLANATION OF FIGURES

FIG. 1. Species of *Myrothecium* after two weeks on potato dextrose agar (20 g. dextrose per liter), 35 cc. medium in each dish, under room conditions.—A, PQMD *185, M. verrucaria,* isolated from an old canvas shoe in England and recorded by Preston as *M. verrucaria;*—B, USDA *1334.2, M. verrucaria,* isolated from stored baled cotton in Washington, D. C. and made by Pope the type of a new species, *Metarrhizium glutinosum;*—C, PQMD *188, M. roridum,* isolated from tomatoes which had been intercepted at the Mexican border by quarantine inspectors of the U. S. Dept. of Agriculture. Magnification approx. ⅔. (By Photographic Dept., Philadelphia Quartermaster Depot.)

FIG. 2. Species of *Myrothecium* after 90 hours incubation on bleached cotton sheeting in the presence of Greathouse Formula A mineral salts solution in a room set at 85° F. and 80 per cent relative humidity—A, Control; —B, PQMD *185, M. verrucaria;*—C, PQMD *188, M. roridum;*—D, USDA *1334.2, M. verrucaria.* Magnification × 9/10. (By Photographic Dept., Philadelphia Quartermaster Depot.)

A NEW GYMNOCARPOUS HETEROBASIDIOMYCETE WITH GASTEROMYCETOUS BASIDIA

DONALD P. ROGERS

(WITH 1 FIGURE)

The hitherto unknown fungus here described grows as a parasite on the hymenium of a minute, inoperculate discomycete superficially resembling the genus *Mollisia*. The host ascocarp, scarcely exceeding half a millimeter in diameter, is barely discernible; the parasite, occurring on only occasional apothecia, is nearly imperceptible to the naked eye, can be detected under the hand-lens only because of its whitish color, and even under the highest magnification of the dissecting binocular appears as no more than a white nap, somewhat convex in profile, covering the gray hymenium of the host. The first collections were the accidental result of bringing the ascomycete into the laboratory, and the few subsequent collections were possible only through persistent search for the host. The fungus must be sought as an alien fuzz on a small discomycete (*Hyaloscypha atomaria*), and is accordingly named *Xenolachne* (from **Ξένος**, a stranger, and Λάχνη, down or fuzz).

The fructification consists of a layer of ovoid or ellipsoid basidia supported on a scanty subiculum of clamp-bearing mycelium, the whole resembling, except for the absence of gelatinous texture, an extremely minute *Sebacina*. The young basidia (FIG. 1, *A–E*) are like those of most species of *Sebacina* except that they develop only one longitudinal septum; and even in this they resemble those of at least one species (unpublished) of *Sebacina,* or those of *Tremella simplex* Jackson & Martin (Martin **1940**: 687) or of some gatherings of *Phlogiotis Helvelloides* (Martin **1936**: 628), or some of the basidia of *Hyaloria Pilacre* (Möller **1895**: 139). Each of the two segments puts out from its apex a tubular or blunt-conic epibasidium (FIG. 1, *F, G*) in no essential different from that of any of the Tremellaceae which possess epibasidia, and the

epibasidium in turn gives rise to a sterigma (FIG. 1, *H–M*). It is the sterigma that is the characteristic and significant organ of *Xenolachne:* instead of being subulate and relatively short, as in the Tremellaceae, it is fantastically elongated (to 35 μ), straight, quite uniform in diameter, and unprecedentedly slender. The sterigmata form the hazy, tenuous fuzz observed under the binocular; or rather, since they are too narrow to present a visible surface at such magnification, it is the light reflected from them, as light is reflected from invisible dust-particles in a sunbeam. None of the sterigmata studied in KOH-phloxine preparations under the oil-immersion lens exceeded 0.2 μ in diameter, and many appeared to be less; in lactophenol, which occasionally is useful to bring out the wall in fungi where KOH seems to show only the protoplast or lumen, the results were no different. The observational error inherent in the estimation of fractions of a division of the ocular scale and in the resolving power of the microscope would make more precise measurement meaningless. The sterigma swells at its tip to form an oblong basidiospore, whose axis is a projection of the axis of the sterigma, and which is without an apiculus (FIG. 1, *L, M*). The spore is abstricted by the breaking, below a septum, of the sterigma, a part of which remains attached to the spore as a false apiculus (FIG. 1, *N–P*); it germinates either by the formation of a sterigma and secondary spore (FIG. 1, *Q–S*) or by mycelium (FIG. 1, *T*), which, as is not surprising in a species with two-spored basidia, has clamp-connections at its earliest septa. At least the majority of the secondary spores are like those of other heterobasidiomycetes in being borne obliquely.

The sterigma, and its relation to the basidiospore, are of a type characteristically gasteromycetous. This is not to say that there is a single basidial type characteristic of the gasteromycetes. But as a correlative of angiocarpy the gasteromycetes have all lost the ability to discharge the spores from the basidia, and with this the structures which in the hymenomycetes make such discharge possible (Rogers **1934**: 168, 179–80). Basidial degeneration has taken various directions in the several groups of gasteromycetes; and one such direction, well shown in a number of the Lycoperdaceae (*e.g.*, many species of *Lycoperdon*), is the development of very long, slender, fragile sterigmata bearing their spores sym-

metrically and setting them free by fracture at or below the point of attachment. This is exactly what occurs in *Xenolachne*.

In this sense, then, and to this degree, *Xenolachne* is gasteromycetous: its basidia show a type of degeneration and a manner of spore-production characteristic of the gasteromycetes. To employ a useful conception and term, *Xenolachne,* like the gasteromycetes, possesses apobasidia.[1] In other respects it shows no resemblance nor evidence of relationship to them. In the gasteromycetes basidial degeneration is the concomitant, and quite probably the result, of angiocarpy; the fructification of *Xenolachne* not

[1] Vuillemin (**1912**: 349) introduced the term *apobasidium* for "des basides secondairement déformées," but mistakenly, as it now appears, applied it to *protobasidia,* a rough equivalent of the better conceived category of *heterobasidia.* In that sense it was of course quite superfluous. Later E. J. Gilbert (**1928**: 225–6) published the group Apobasidiomycetes, explicitly limited to those Autobasidiomycetes in which "les spores sont terminales et symétriques à l'extrêmité et dans le prolongement axial des stérigmates." As a formal designation of a taxonomic group, Apobasidiomycetes is exactly synonymous with Gasteromycetes, and therefore both superfluous and invalid. In addition, Gilbert used the term *apobasidium* for basidia forming sterigmata and spores in the relation just described. It is this sense of the term which seems to have been accepted (Snell **1936**: 12; Ainsworth & Bisby **1945**: 39). It would be difficult to coin a name more suitable for a basidium deprived of one of its primary functions, that of spore-discharge; the appropriateness is not diminished by Gilbert's denial of phyletic implications. It should, however, be noted that while Gilbert clearly intended the term to refer to all basidia of gasteromycetes, his description excludes those forms in which the sterigma has been not modified but lost—that is, those, like *Melanogaster,* with sessile spores.

It is now proposed that *apobasidium* be used with the phyletic connotation stated by Vuillemin, for degenerate basidial types; that it be applied not only to such basidia as those described by Gilbert but also (as he apparently intended) to those in which the sterigmata have disappeared; that it be not limited to the subclass Homobasidiomycetes (nor to the Autobasidiomycetes), but used also for certain basidia among the Heterobasidiomycetes; and that it be employed not in antithesis to *heterobasidium* and *homobasidium* but as a complementary term. Accordingly *Xenolachne* would possess heterobasidia which are also apobasidia, and *Lycoperdon* homobasidia which are also apobasidia. Such an emendation of the sense of the term seems permissible, and by it *apobasidium* is referred to that morphological entity which stands in need of a name. A definition is suggested: **Apobasidium**—a basidium whose basidiospores are not apiculate nor borne obliquely on the sterigmata nor forcibly discharged. EXAMPLES—gasteromycetes, Sirobasidiaceae, Hyaloriaceae, Phleogenaceae, most (if not all) Ustilaginales. There is some additional discussion by the writer (Rogers **1934**: 166–8) of the three levels of basidial evolution (though the apobasidium is not so named).

only has no peridium, but even lacks the gelatinous hymenial matrix in which in most Tremellales the basidia are more or less completely immersed. As a group, and especially among their least modified forms (*Endoptychum, Montagnea*), the gasteromycetes are conspicuously xeromorphic, and their origin is probably a response to the limitations of an arid habitat; *Xenolachne,* like other delicate heterobasidiomycetes, develops its fructifications under conditions of continuous high humidity—on the lower surface of water-soaked logs, in mesophytic forests, during wet weather—and is, in fact, more readily damaged by exposure to the air of the laboratory than are most related fungi. The ecological significance of its apobasidia is entirely obscure. It is to be supposed that the sterigmata, instead of discharging their spores, retain them until separation is effected by some mechanical disturbance. There is some temptation to guess at distribution by small arthropods, or at a mode of dissemination by which the basidiospores of the parasite are carried along with the ascospores of the host when the latter are discharged. But no evidence exists of arthropod agency, and the apothecia of the host appear to be too heavily infested to permit normal formation of ascospores, let alone their discharge through the *Xenolachne* fructification. No more is known than that a completely gymnocarpous form has here reverted to an essentially conidial manner of dissemination of basidiospores.

Xenolachne is unique also in the presence of both indubitable epibasidia and grotesquely elongated sterigmata. In heterobasidiomycetes with normal—that is, subulate—sterigmata the sterigma, though distinct enough, is not always conspicuous as a separate entity. In this fungus neither the *Sebacina*-like epibasidium nor the *Lycoperdon*-like sterigma can be overlooked; and to treat them as manifestations of a single structural entity is as little satisfactory as to do so for, say, the carpogonial base and the trichogyne of one of the Florideae. Here, as perhaps nowhere else, the distinctness of the two organs is obvious.

The ovate, longitudinally septate basidia of *Xenolachne* are such as to place it in that phyletic series of the Basidiomycetes characterized by hypobasidial meiosis (Rogers **1934**: 165), and in that division of the Tremellales which includes the families Tremella-

ceae, Sirobasidiaceae, and Hyaloriaceae (Martin **1941**: 23; **1944**: 11; in Ainsworth & Bisby **1945**: 406). Of these families it is clearly foreign to the Sirobasidiaceae, in which the basidia are catenate and the spores sessile. Since *Xenolachne* is gymnocarpous, it might be assigned to the Tremellaceae, and more particularly, because of its resupinate fructification, to a position near *Sebacina*. By existing keys and descriptions it would be excluded from the Hyaloriaceae, whose single genus is ostensibly angiocarpous and unquestionably stipitate. But the presence or absence of a stipe has not usually been considered to be of sufficient importance to constitute by itself the basis for the assignment of fungi to different families; and since abundant cases are known where fungi differing on this point agree on all others of any importance, it is apparent that here taxonomic habit is supported by what is known of comparative morphology or can be inferred concerning phyletic relationship. The difference between gymnocarpous and angiocarpous development is of considerably greater weight. In *Hyaloria*, however, the basidia are merely considerably overtopped by the paraphyses and immersed in a layer of gelatinous material to which the paraphyses give rise (Möller **1895**: 137–40, 173; pl. 1, *fig*. 3; pl. 5, *fig*. 37; Martin **1937**: 620–4; *fig*. 10–27); there is nothing approaching a peridium and, except that the spores are not formed at the surface, nothing in the organization of the fruiting layer to distinguish it from those species of *Sebacina* and *Tremella* which have long and abundant paraphyses (cf. the figures of *Sebacina epigaea* given by McGuire, **1941**: pl. 1, *fig*. 2, 4). This published evidence is abundantly supported by study of specimens of the fungi cited. The great taxonomic significance of angiocarpous development, at least among basidiomycetes, lies not in angiocarpy itself, but in the profound changes in structure and behavior of fruiting organs that accompany, and have apparently been induced by, angiocarpy. The significant character of *Hyaloria* is not that the hymenium is enclosed (it is not), nor even that the basidia are embedded in gelatinized paraphyses (those of *S. epigaea* and of other Tremellaceae are more deeply embedded), but that its spores are terminal and symmetrical on the sterigmata and are freed as are conidia or the spores of gasteromycetes. In this respect *Hyaloria* does not differ

from *Xenolachne*. The one is stipitate and the other resupinate; the one lacks epibasidia and the other has retained them; the one develops its spores among gelatinized paraphyses and the other in the air. But because both have tremelloid hypobasidia (and presumably an origin from the Tremellaceae) and both have symmetrical spores separated by fracture from capillary sterigmata, the two genera appear to be closely related (though not necessarily in series), and are accordingly assigned to the one family. Möller's description of the Hyaloriaceae reads: "Der angiokarpe Fruchtkörpertypus . . . er fand sich abermals vor, ausgestattet mit Basidien der Tremella-Form." As already noted, neither Möller's account nor his figures support the assertion that *Hyaloria* is angiocarpous; that assertion seems to be one of Möller's attempts to display his newly discovered genera as the fulfilment of Brefeld's prophecies concerning the structural diversities of the basidiomycetes (Möller **1895**: 143; Martin **1934**: 144). To eliminate that error, as well as to permit the inclusion of the additional genus, that diagnosis has been emended, and the character of angiocarpy replaced by the essential one of the relation of spore to sterigma.

HYALORIACEAE Möll., Protobas. (Schimper, Bot. Mittheil. Troper **8**:) 137, 143. 1895, emend.

Hypobasidia longitudinally septate; basidiospores without apiculi, borne on filiform sterigmata, with respect to which they are symmetrical and coaxial.

Genera: *Hyaloria* (type), *Xenolachne*.

Xenolachne gen. nov. Hyaloriacearum

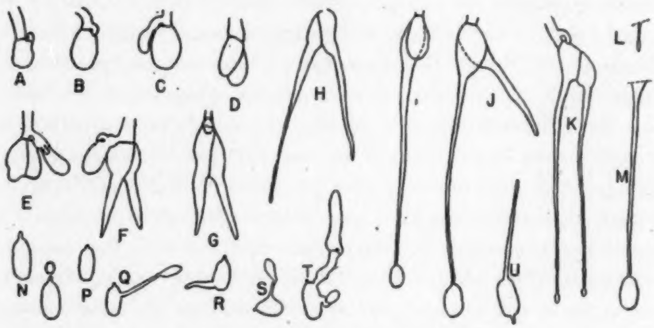
Fructificatio resupinata; hypobasidia longitudinaliter septata, eorum segmentis tot epibasidia tubuliformia gerentibus, sterigmatibus filiformibus, longitudine variis; sporae recte in sterigmatibus porrectae, sine apiculo genuino, sterigmatis apicem sicut apiculum retinentes, per repetitionem germinantes.

Basidiocarp resupinate; hypobasidia longitudinally septate, each segment bearing a tubular epibasidium continued in a sterigma of variable length; basidiospore without a proper apiculus, borne symmetrically upon its sterigma, and after abstriction retaining the terminal portion of the sterigma as a false apiculus, germinating by repetition.

Type: *X. flagellifera*.

Xenolachne flagellifera sp. nov.

Basidiocarpium album, minutissime pilosum; hyphae nodoso-septatae, 2 μ diam.; hypobasidia elongato-ovata vel ellipsoidea, 7.5–10 × 5–6 μ, longitudinaliter uniseptata, epibasidia duo gerentia 2–3 μ diam., 10 μ long., in sterigmata filiformia ad 35 μ long., 0.2 μ crass., producta; sporae oblongo-cylindraceae, apice sterigmatis ornatae, 5.5–8 × 2.5–3.5 μ, per repetitionem, vel in mycelium zygodesmatibus ornatum, germinantes.



EXPLANATION OF FIGURE

(All drawings made with camera lucida, and reproduced at approximately 1000 ×.)

FIG. 1. *Xenolachne flagellifera.* A–D, young basidia; E–G, development of epibasidia; H–K, basidia with sterigmata and developing spores; L, tip of sterigma with very young spore (Note: in this figure alone the relative diameter of the sterigma is accurately represented.); M, mature spore, and septum in sterigma; N–P, abstricted spores; Q–S, germination by secondary spores (In S the germinating spore is probably itself a secondary spore.); T, germination by mycelium; U, spore germinating by a sterigma like that of the basidium.

Fructification an almost imperceptible white fuzz, under the binocular (40 ×) a delicate, glistening, snow-white pile or nap composed of very slender threads tipped by minute swellings, collapsing on drying; hyphae distinct, not gelatinized, with clamps throughout, 2 μ in diameter, a few emergent, obtuse, cystidioid, 1.5–2 μ in diameter; basidia subtended by proliferating clamps; hypobasidia elongate-ovate or -ellipsoid, 7.5–10 × 5–6 μ (or rarely subfusiform, –20 μ long), with one longitudinal septum, bearing two subcylindric or tapering epibasidia 2–3 μ in diameter and about 10 μ long, each more or less abruptly attenuated to a capillary flagelliform sterigma under 0.2 μ in diameter, up to 35 μ long; spores borne symmetrically on the sterigmata, without apiculi,

oblong-cylindric, 5.5–8 × 2.5–3.5 μ, tipped by the end of the sterigma, germinating by repetition or by a clamp-bearing mycelium.

Parasitic on the hymenium of *Hyaloscypha atomaria* (Starb.) Nannf. growing on the lower side of damp logs of *Libocedrus decurrens, Pinus Strobus,* and *Pseudotsuga Taxifolia.*

SPECIMENS EXAMINED: MASSACHUSETTS: N. branch of Mill R., Springfield, IX.23.43, *A. M. & D. P. R. 1040.* OREGON: Umpqua valley E. of Reedsport, X.22.38, *A. M. & D. P. R. 487,* **type**, and *490* [2]*;* Lorane, X.22.38, *M. S. Doty,* in *D. P. R. 1039;* Alsea Rd. S. of Philomath, XII.13.38, *A. M. & D. P. R. 1038.*

For assistance with this paper I am indebted to Dr. M. S. Doty of Northwestern University, Miss Edith K. Cash of the United States Department of Agriculture, Professor G. W. Martin of the University of Iowa, and Dr. S. M. Zeller of Oregon State College. Dr. Doty supplied one of the original collections of *Xenolachne;* Miss Cash identified the ascomycetous host; Dr. Martin supplied sectioned and whole material of *Hyaloria* on which the comparison with *Xenolachne* was based; in the laboratory and in the field Dr. Zeller provided a unique introduction to the many groups of gasteromycetes with which the Northwest forests and his studies have enriched the science of mycology. Dr. Zeller and Dr. Martin have very kindly criticized the manuscript.

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WHAT IS AN ANTIBIOTIC OR AN ANTIBIOTIC SUBSTANCE? *

SELMAN A. WAKSMAN [1]

With the introduction, in 1941, of penicillin as an important chemotherapeutic agent, and with the isolation from cultures of different micro-organisms of a rapidly increasing number of new chemical substances found to possess similar antibacterial and other antimicrobial properties, it became apparent that a new name was required, which would include these and similar compounds.

A request for the submittal of such a name was addressed to the writer in July, 1941, by Dr. A. Flynn, editor of Biological Abstracts. After giving this matter considerable thought, the writer concluded that the terms *antibiotic* and *antibiotic substance,* which previously had been used in a rather loose sense, might well be restricted to a specific application. It was felt that the words *antagonism* and *antagonistic action* would be best reserved for the antimicrobial activities of the living systems as a whole, whereas the newly selected words should be used to designate the action of the *chemical agents,* produced by micro-organisms and possibly other living bodies, which were responsible for these antimicrobial effects.

The use of the words "antibiosis" and "antibiotic substance" to designate antiliving processes in a very broad sense is found in the older biological literature as well as in many dictionaries. The scientific use of the word "antibiosis" dates from the concept first expressed, in 1889, by Vuillemin (**5**) in the following terms: "one creature destroying the life of another in order to sustain its own . . . one being in unrestricted opposition to the life of the other"; the active participant was named "antibiote." This concept was also held by Marshal Ward (**13**), who applied the term "antibiosis" to an association of organisms whereby one injures the

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other, "as exemplified by many parasites." Webster's New International Dictionary defines "antibiotic" as "tending to prevent, injure, or destroy life"; other dictionaries define the term as "injurious to or destructive to living matter."

To indicate the limited use of the term prior to 1941 and the confusion which frequently was brought about when the word was applied to microbiological reactions, one has only to consider the classical book of Papacostas and Gaté (**4**), who summarized, in 1928, the knowledge on microbial associations. These authors proposed that the word "antibiosis" be defined as follows: "When in a culture where several bacterial species occur or are intentionally mixed, one organism exerts an injurious effect upon another, it is antibiosis." The word "antibiosis" was further limited to the activities of one organism upon another *in vitro* or in the test tube as opposed to "antagonism" which would apply to the action of one organism upon another *in vivo* or in the animal body. The word "antibiosis" was thus applied to mixed cultures and the word "antagonism" to mixed infections. "Reciprocal antibiosis" was differentiated from "unilateral antibiosis" and " vital antibiosis" from "functional antibiosis." Antibiotic microbes were believed to act upon other microbes indirectly, subtracting oxygen but also directly by means of a substance which they produce. To indicate the confusion thus involved, it is sufficient to quote the following: "An antibiotic microbe *in vitro* may sometimes also be an antagonistic microbe *in vivo.*" The chemical thus produced is spoken of as a "toxin," and nowhere is it indicated that the same substance is involved whether the action takes place *in vitro* or *in vivo*.

Various designations were thus applied to the specific chemical substances, possessing characteristic antibacterial properties and produced by antagonistic micro-organisms (**6**). It is sufficient to mention some of the common terms: "bacterial toxins," "toxic substances," "staling products," "antagonistic substances," "antibiotic substances," "inhibitory substances," "bacteriolytic substances," "bacteriostatic substances," "antibacterial substances," "antimicrobial substances," "active substances," and "lethal principles."

The terms "antibiotic" and "antibiotic agent" were first used in the present sense by Waksman and his collaborators in several

papers published or written in 1942 (**9, 10, 11, 12**). Since 1942 the terms have come into general use. The word "antibiotic" was defined (**7, 8**) as "inhibiting the growth or the metabolic activities of bacteria and other micro-organisms by a chemical substance of microbial origin." An "antibiotic substance" or an "antibiotic" was defined as "a chemical substance, of microbial origin, that possesses antibiotic properties."

Oxford (**3**), in a review of the subject of "antibiotic substances," characterized antibiotics as "soluble" and as produced from a harmless constituent of a medium. Three qualifications were attached to this definition; namely (a) an antibiotic substance shall have been isolated and tested in a pure state; (b) algae and higher plants are excluded as producers of antibiotic substances; and (c) the smallest concentration of the antibiotic substances in the medium producing a measurable effect upon the inhibited organism *in vitro* shall be of the order of 50 p.p.m. or less. Neither Oxford's definition nor the qualifications attached to it are in accord with the original definition nor with the present use of the term "antibiotic." For example, certain substances, such as tyrothricin, widely recognized as antibiotics, are definitely cell constituents and not products of a harmless constituent of the medium. If a substance is to be recognized only after it has been isolated in a pure state, what would have become of penicillin, streptomycin, and similar antibiotics, which not only were studied but were used for chemotherapeutic purposes before they were purified? Again, to delimit the concentration of an antibiotic in the medium before the antibiotic has been recognized as such would not always be justified.

Several suggestions have been made that the concept of the word "antibiotic" be enlarged, and that substances falling within the class of antibiotics be further subdivided. These subdivisions would be based upon the origin of the substance or upon its mode of action.

Chain and Florey (**1**), for example, suggested that all antibiotic substances be divided into two groups: (1) antibiotics that react with protoplasmic constituents and kill both bacterial and animal cells, in a manner comparable to that of "antiseptics" (in other words, those antibiotics that are toxic to the animal body); and

(2) antibiotics that react with substances having a specific significance in the bacterial cell only (since some of the latter substances are largely growth-inhibiting, they were designated as "bacteriostatics"). In his later work, however, Florey (2) apparently dropped this plan of subdivision.

Wingo (**14**) suggested that the term "antibiotic" be enlarged to include, in addition to microbial products, other substances that possess antibacterial properties. The group of antibiotics would thus be subdivided into "mycoantibiotics" (products of bacteria and fungi), "chemoantibiotics" (synthetic compounds), "actinoantibiotics" (radiant energy). Were such a division justified, one would have to add to this list "phytoantibiotics" (products of green algae and higher plants) and "zoöantibiotics" (lysozyme, erythrin, and other animal products). This enlarged concept is hardly desirable, for various obvious reasons.

It is quite evident that the present widespread use of the term "antibiotic" in the sense first implied by Waksman and his collaborators has evoked some philological discussion. It may be advisable, therefore, in the interest of semantics, to amplify the earlier definition as follows:

An antibiotic is a chemical substance, produced by micro-organisms, which has the capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms. The action of an antibiotic against micro-organisms is selective in nature, some organisms being affected and others not at all or only to a limited degree; each antibiotic is thus characterized by a specific antimicrobial spectrum. The selective action of an antibiotic is also manifested against microbial vs. host cells. Antibiotics vary greatly in their physical and chemical properties and in their toxicity to animals. Because of these characteristics, some antibiotics have remarkable chemotherapeutic potentialities and can be used for the control of various microbial infections in man and in animals.

In accordance with this definition, plant products possessing antimicrobial properties would be eliminated from the group of antibiotics. They can either be designated as "antibiotic-like substances" or as "plant antibiotics," or perhaps even better as "phytoncides," a term proposed for this group of substances. Of course,

care will have to be taken not to confuse this term with that of "phytocides," used to designate materials that act injuriously upon plants.

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SOME ASPECTS OF PENICILLIN PRODUCTION BY ASPERGILLUS NIDULANS [1]

EUGENE L. DULANEY [2]

A number of *Aspergillus* species have been shown to produce penicillin. This list of species has already been reviewed by Raper (**9**). In this paper he also noted that the ability to produce penicillin is more general in the genus *Aspergillus* than in the genus *Penicillium*. Penicillin producing strains, however, do appear to be commonly found within the *Aspergillus flavus* group. It seems that this ability is also commonly found within various species of the *Aspergillus nidulans* group (**1**).

Aside from the fact that these *Aspergilli* produce penicillin little more is known. The relatively low yields obtained from them indicate that there is little hope for them in commercial competition with *Penicillium* strains now used in production. It would be interesting, nevertheless, to learn something of penicillin production by these organisms. For this purpose a strain of *Aspergillus nidulans* was chosen.

METHODS

The strain of *Aspergillus nidulans* (Eidam) Wint. used in this research was isolated from compost. Its identification as *A. nidulans* was confirmed by K. B. Raper.

Spores from six day old honey peptone agar cultures, grown at 24° C., were used as inoculum for setting up fermentations. The spores were suspended in sterile distilled water, and one milliliter of the suspension added to each flask. The fermentation medium contained the following ingredients in grams per liter of distilled

[1] Portion of a thesis submitted to the graduate school of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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water: lactose 20.0 gr., corn steep solids (Amaizo spray dried) 20.0 gr., sodium nitrate 3.0 gr., potassium phosphate monobasic 0.5 gr., magnesium sulphate·$7H_2O$ 0.25 gr., zinc sulphate·$7H_2O$ 0.04 gr. Substitutes for the lactose and corn steep were used in certain experiments. The medium was dispensed in 100 ml. portions into 500 ml. Erlenmeyer flasks. Experiments showed that when lactose was the carbon source near-maximum production was obtained with this depth of medium. After sterilization and inoculation, the flasks were placed on a reciprocal type shaker. The speed of the shaker was 92 cycles per minute; each cycle consisted of an eight inch movement, four inches forward and four inches backward. The incubation temperature was 24° C.

The cylinder plate method, which has been adequately described (2), was used in the assays throughout the experiments.

The changes in mycelium weight, pH, and penicillin production occurring during fermentation were followed. In addition, changes in the concentration of lactose, ammonium nitrogen, nitrate nitrogen, and organic nitrogen, were determined and correlated with the other changes. The methods for these determinations are given in the appropriate section.

EXPERIMENTAL DATA

Evidence for Penicillin Formation—Proof that an antibiotic is or is not penicillin would come with chemical purification and characterization of the substance. The active substance produced by *Aspergillus nidulans* has not been chemically characterized, but other evidence indicates that the antibiotic activity is due to penicillin.

Aspergillus nidulans and *Penicillium notatum* strain 832 were grown on the lactose corn steep medium plus salts. Both stationary and submerged fermentations were employed. When the antibiotic titers were high the mycelium was filtered off and a series of tests run on the filtrates. The tests and results are given below.

1. *Inactivation by penicillinase.* Filtrates were adjusted to pH 6.7. Penicillinase completely inactivated the filtrates after incubation for three hours at 37° C.

2. *Method of extraction.* The active substance from metabolism solution filtrates of *A. nidulans* could be extracted by a method commonly used for extraction of penicillin.

3. *Inactivation by hydroxylamine.* After incubation at 28° C. for four hours, the metabolism solution filtrates were completely inactivated by hydroxylamine.

4. *Inactivation by copper sulphate.* At a concentration which did not inhibit the test bacterium, copper sulphate inactivated the filtrates after four hours incubation at 28° C.

5. *Instability at low and high pH.* Portions of the filtrates were adjusted to pH 2.5 with 1 N HCl and other portions were adjusted to pH 10 with 1 N NaOH. After incubation at 28° C. for four hours the filtrates were readjusted to approximate neutrality and assayed. In each case the antibiotic activity had completely disappeared.

6. *Solubility.* Filtrates were adjusted to pH 2.5 with 1 N HCl and extracted with various organic solvents. The antibiotic activity from *A. nidulans* filtrates and *P. notatum* filtrates showed the same solubilities.

7. *Antibacterial activity.* Filtrates from the two organisms were tested against fourteen test bacteria. In so far as it was tested the antibacterial activity of the two molds was identical. Included among the fourteen test bacteria was a penicillin resistant *Staphylococcus aureus* which was uninhibited by either organism.

As can be seen from the above tests the reaction of the metabolism solution filtrates from the two molds was quite comparable. This comparison would indicate that the antibiotic activity of the filtrates is due to similar types of compounds. It is known that *Penicillium notatum* strain 832 produces penicillin(s) when grown under the above conditions, and it would follow that *Aspergillus nidulans* is also producing penicillin(s).

Carbon Sources—The fermentation media used for the production of penicillin by *Penicillium notatum* or *P. chrysogenum* frequently contain lactose as the carbon source. The fermentation medium used in the preliminary work with *Aspergillus nidulans* also contained lactose at a 2 per cent level. The medium used for this preliminary work, however, had been chosen because it was known to support penicillin production by the above *Penicillia.* Due to this fact it appeared quite possible that other carbon sources could support better yields when *A. nidulans* was the producing organism. With this in mind nineteen different carbon sources

TABLE 1

CARBON SOURCES

Carbon Source	Yield in mm. per day						
	3	4	5	6	7	8	9
whole ground corn	25.8	27.4	30.3	31.1	32.1	32.0	32.4
whole ground oats	18.0	24.0	29.1	29.4	32.0	32.7	32.1
whole ground wheat	22.2	25.8	28.2	29.0	32.1	31.0	30.2
whole ground barley	24.3	26.0	30.0	30.5	31.4	30.8	30.6
glycerine	26.7	27.5	28.8	27.7	29.8	29.8	29.4
d-xylose	0.0	0.0	0.0	18.4	24.0	25.7	27.3
d-arabinose	0.0	21.2	27.0	27.7	26.6	25.0	22.0
dextrose	18.4	21.1	24.7	26.5	24.5	28.1	27.7
mannite	27.7	28.3	26.8	26.6	29.1	28.9	28.4
d-galactose	19.4	22.7	25.9	28.1	31.0	29.9	29.3
honey	17.0	20.7	25.1	27.7	28.7	28.3	27.6
sucrose	16.6	20.0	24.8	25.3	28.3	27.7	28.0
maltose	19.7	22.7	23.7	25.9	28.7	28.1	27.9
lactose	25.8	27.0	27.3	28.3	30.5	30.0	29.7
brown sugar	11.7	17.6	20.6	24.7	26.3	27.0	
molasses	20.1	24.5	26.9	28.0	29.1	27.1	26.8
soluble starch	22.7	26.2	26.6	28.2	28.6	28.3	27.9
dextrin	21.3	22.1	26.7	27.2	28.9	28.6	27.2
sodium glycerophosphate	27.7	27.7	26.9	27.7	28.7	28.2	27.0
whey powder	25.1	27.4	28.6	28.3	27.2		

were substituted for lactose in the lactose corn steep medium plus salts. The results are given in Table 1.

The whole ground grains supported the best yields. There is no certainty, however, that this is due solely to the added carbon. Galactose and lactose were the best of the sugars tried, though some of the others supported yields which were almost as high. Use of glycerine and sodium glycerophosphate resulted in yields lower than those supported by lactose but the potencies came up much faster. This was also true for mannite. The production of penicillin on the various carbon substrates could be correlated with the pH changes. For example, use of d-xylose and d-arabinose, which resulted in low early potencies, also resulted in a slow rise in pH.

It is quite possible that different results would have been obtained if a higher rate of aeration had been used as well as a higher level of carbohydrate. The effect of aeration upon penicillin production when various carbohydrates are used has already been discussed (**4**). Indeed, the superiority of lactose for penicillin production, under the conditions frequently employed, appears to be

due to the fact that the oxygen supply is near the oxygen demand for this sugar (**5**).

In order to investigate the effect of aeration and concentration a number of carbohydrates were chosen. Three of these, glycerine, sodium glycerophosphate, and mannite, resulted in an early rise in potency. Two others, whole ground corn and galactose, supported good yields but resulted in slow increase of penicillin. Lactose was also included. These materials were employed at 4 per cent concentration. Aeration was increased by using 50 ml. amounts of the fermentation medium in the Erlenmeyer flasks as compared with the 100 ml. previously used. The results are shown in Table 2.

TABLE 2

CARBON SOURCES

Carbon Source	Yield in mm. per day					
	3	4	5	6	7	8
whole ground corn						
4% in 100 ml.	21.6	25.9	25.6	25.2	25.2	
4% in 50 ml.	18.4	24.7	27.2	29.8	31.8	34.0
sodium glycerophosphate						
4% in 100 ml.	27.3	27.0	27.1	25.7	23.8	
4% in 50 ml.	26.5	26.6	26.4	24.8	22.3	
mannite						
4% in 100 ml.	21.0	27.1	28.0	26.0	25.9	
4% in 50 ml.	26.0	27.7	28.8	26.0		
glycerine						
4% in 100 ml.	15.9	25.9	27.9	28.5	26.8	
4% in 50 ml.	23.6	26.8	28.5	27.2	26.4	
galactose						
4% in 100 ml.	0.0	18.4	18.7	24.9	25.6	29.4
4% in 50 ml.	23.6	25.7	29.2	27.9	31.5	
lactose						
4% in 100 ml.	21.7	27.7	29.8	27.7	27.7	
4% in 50 ml.	24.7	27.7	28.6	26.2	25.7	

In comparably aerated fermentations none of the carbon sources at 4 per cent level resulted in greater yields than the same carbon source supported at 2 per cent level. In fact, in each case the penicillin level was lower. Increased aeration did not markedly

increase the yields obtained with sodium glycerophosphate, glycerine, mannite, or lactose. The higher rate of aeration did result in better yields with whole ground corn and galactose, though with the former the peak came considerably later at the higher aeration. With some of the carbohydrates, an increased aeration exerts a striking effect on the rise in penicillin production. This effect is particularly noticeable when glycerine and galactose are used. It is somewhat noticeable with mannite and lactose.

As had been noted the best of the twenty substances tried were the whole ground grains, but the stimulation by these substances may not necessarily be due to the added carbon. If the level of corn steep is raised to 4 per cent, lactose supports yields higher than those obtained with the whole ground grains. Lactose was chosen over the whole ground grains for later work, not only because of this, but also because use of the whole ground grains in addition to corn steep would have resulted in a very complex medium.

Corn Steep Substitutes—The discovery (**6**) of the efficacy of corn steep liquor in increasing yields is one of the landmarks in the history of penicillin. The function of corn steep in penicillin production is varied. One of the reasons for its usefulness, however, appears to be due to the precursors it contains. In the following work none of the known precursors was added, but a number of organic materials were investigated as substitutes for corn steep. The supplements were added at 2 per cent levels and compared with corn steep (Amaizo spray dried) at 2 per cent, 4 per cent, and 6 per cent levels. The results are shown in Table 3.

Soy bean meal was the best of the substitutes tried. When grass juice powder was used as a substrate, however, the yields were only slightly lower. It is well known that materials such as corn steep and soy bean expeller meal vary greatly. It is quite possible that different results would have been obtained if other lots of these materials had been used. Striking results were obtained with corn steep solids at different concentrations. Highest yields were obtained with corn steep at a 4 per cent level. The 6 per cent level supported yields slightly better than those obtained with a 2 per cent level though the potencies came up more slowly at the higher concentration. This could perhaps be due to the fact that the

TABLE 3

CORN STEEP SUBSTITUTES

Substitute	Yield in mm. per day					
	3	4	5	6	7	8
yeast extract	24.8	25.8	24.6	25.3	25.1	
beef extract	20.6	20.9	21.1	19.3	19.7	
malt extract	12.2	17.9	18.9	20.2	18.4	
Bacto peptone	22.0	20.9	20.5	17.2	12.1	
Parke-Davis peptone	20.3	23.7	23.5	21.4	16.8	
Wilson peptone	20.3	21.7	22.4	20.8	17.1	
dry potatoes	19.9	22.0	26.3	26.4	26.8	24.4
liver powder 1:20	14.6	22.1	26.2	27.4	27.6	24.6
soy bean meal	26.5	28.1	28.4	29.3	28.0	25.6
grass juice powder	24.2	26.4	27.9	28.5	27.4	
fish meal	24.4	26.4	27.3	27.7	27.7	25.1
asparagus butt juice	0.0	0.0	13.4	19.2	20.6	18.9
corn steep solids (2%)	23.1	25.9	29.9	30.7	30.7	27.9
corn steep solids (4%)	20.1	28.3	32.9	33.6	34.2	
corn steep solids (6%)	0.0	18.3	21.7	28.0	31.8	30.1

fermentation remained acid too long as a result of the high concentration of lactic acid in the corn steep.

Results, as those given above, are difficult to evaluate if only the *Staphylococcus aureus* assay yields are given. Various organic supplements contain different amounts of the various penicillin precursors. If the metabolism solutions, from fermentations in which these organic supplements are used, are not analyzed for the changing content of the different penicillins it is difficult to determine the worth of the material added.

In addition the corn steep apparently contains the salts and heavy metals necessary for growth and penicillin production by *Aspergillus nidulans*. On a medium containing 2 per cent lactose and 4 per cent corn steep, yields were as good or better when salts were omitted than when they were added.

Chemical Changes Occurring in the Medium During Fermentation—The chemical changes occurring in the medium during fermentation were determined daily on composite samples obtained by combining the contents of three Erlenmeyer flasks. The mycelium was filtered off, dried at 80° C. for 48 hours, then weighed. The pH of the filtrate was determined with a glass electrode and the penicillin content measured by the cylinder plate method. Lactose

content of the filtrate was determined by Somogyi's copper reduction method after the samples were hydrolyzed in 1 N HCl by autoclaving at 15 lbs. pressure for 30 minutes; readings were made from a curve based on pure lactose. The total nitrogen was determined by the Kjeldahl method. Nitrate nitrogen was determined by reduction to ammonia in the presence of DeVarda's alloy in hot alkaline solution, and ammonium nitrogen by alkaline cold aeration for four hours. The total organic nitrogen is Kjeldahl nitrogen minus ammonium nitrogen.

The changes occurring during the fermentation are shown in Table 4.

TABLE 4

CHEMICAL CHANGES OCCURRING IN THE MEDIUM DURING FERMENTATION

Factor Determined	0	1	2	3	4	5	6	7	8
Lactose per cent remaining	100.0	97.0	83.4	68.5	51.0	33.0	30.0	18.0	18.0
NO_3-N per cent remaining	100.0	71.0	60.5	63.8	72.2	38.8	32.9	33.5	33.5
NH_3-N per cent remaining	100.0	6.6	8.4	19.2	150.0	191.0	231.0	200.0	178.0
Organic N per cent remaining	100.0	98.2	98.2	92.9	81.3	50.9	56.3	55.4	55.0
Mycelium mg./ml.	0.0	0.8	1.3	24.3	49.0	56.5	56.5	56.5	50.0
Penicillin zones in mm.	0.0	0.0	11.0	23.3	34.0	36.0	38.5	37.0	37.0
pH	3.5	3.8	4.4	6.1	7.1	7.2	6.9	7.8	7.8

Changes in pH. The pH rises slowly the first two days, then rapidly the third and fourth days. From the fourth day on a pH plateau between 7.0 and 8.0 is maintained. It may also be noted that coincident with the increase in pH there is a large increase in the dry weight of mycelium and a rise in penicillin production.

The rise in pH may be attributed to several factors. Ammonia accumulation appears to be one of these. The ammonia apparently arises from deamination of organic nitrogen compounds of the corn steep as well as from reduction of nitrate nitrogen. Another contributing factor to this pH rise is probably the utilization of lactic acid supplied in the corn steep. In addition the utilization of ni-

trate ions with the subsequent release of sodium ions would contribute to this pH rise.

Changes in the Dry Weight of Mycelium. Most of the mycelium is formed during the first four days coincident with the increase in pH. After the pH plateau is reached there is little increase in mycelium weight. This is in line with what has been found with *Penicillium notatum*, when grown in the same medium, where it has been shown that mycelial growth occurs rapidly at low pH values while little increase in dry weight occurs at neutrality (7). In the fermentations carried out with *Aspergillus nidulans*, the mycelial carbon appears to come from lactose as well as the corn steep. Whereas the lactose is utilized quite rapidly from the second until the sixth day, it is not exhausted at the end of eight days. The rapid increase in mycelium coincident with the increase in ammonium nitrogen and only a slight decline in nitrate nitrogen indicates that the mycelial nitrogen comes from the organic nitrogen compounds of the corn steep.

From four to seven days there is little apparent gain in mycelium weight; autolysis, as revealed by dry weight measurements, begins at seven days. Autolysis may occur earlier, however, a possibility which is supported by the fact that the total organic nitrogen increases somewhat on the sixth day and remains relatively unchanged throughout the eighth day. This increase in organic nitrogen on the sixth day could be due to organic nitrogen being liberated by the autolyzing mycelium.

Changes in Penicillin Content of the Medium. Rise in penicillin production starts about the second day and increases rapidly until the fourth day at which time it levels off. The greatest penicillin production comes at the time of the pH plateau, with the maximum appearing slightly before the apparent autolysis of the mycelium. This may indicate release of penicillin by autolysis, a possibility which was investigated as follows. The metabolism solution was decanted from seven day old fermentations, the mycelium of which was ready to autolyze. Upon assay this metabolism solution produced zones of 32.0 mm. Sterile phosphate buffer at pH 7.0 was added to each flask and the pellets thoroughly washed. This buffer was then decanted and replaced with 100 ml. of fresh sterile buffer. The flasks were replaced on

the shaker and assayed at 24 and 48 hours. After 24 hours, the solution produced zones of 29.0 mm. and after 48 hours, zones of 33.0 mm. After 48 hours, then, the autolyzing mycelium had liberated more penicillin than was contained in the seven day old metabolism solution.

DISCUSSION

Metabolism solutions of *Penicillium notatum* and *P. chrysogenum* have been shown to contain a number of penicillins. Some of these have been chemically characterized, and it is quite possible that other as yet unidentified ones exist. As noted before (**1**) the type of penicillin being produced by *Aspergillus nidulans* has not been determined. Two penicillins produced by other species of *Aspergillus,* however, have been identified. Gigantic acid, produced by *A. giganteus,* is pentylpenicillin (**8**), whereas flavicidin (**3**), a metabolic product of *A. flavus,* is Δ3-pentenylpenicillin. The latter organism also appears to produce some G penicillin. There is no reason to assume that the antibiotic activity produced by *A. nidulans* is due to only one type of penicillin. It is quite possible that the metabolism solution will be found to contain several penicillins depending upon the constituents of the medium as well as upon the strain used. The possibility also exists that *A. nidulans* is producing an antibiotic that has not as yet been chemically or biologically identified. Whatever the situation, the highest yield obtained in terms of penicillin units, when the above fermentation conditions were employed, was 20 u/ml. It has been shown, however, that the addition of a penicillin precursor such as a phenyl acetyl derivative will greatly stimulate production by this species (**1**). The synthetic medium of Stone and Farrell (**10**) also supported yields equivalent to those obtained on the unsupplemented lactose corn steep medium (**1**).

Yields could probably be increased by means of induced mutation and strain selection. Preliminary investigation, however, indicates that strain selection alone does not offer much hope. Observations of 1,000 monoconidial lines showed the organism to be quite stable as to morphology and antibiotic production. This might not be the case if other isolates from nature were used. In addition *A. nidulans* readily produces perithecia in culture. It is

quite possible that single ascospore selection would result in greater variability. Breeding for greater productivity would be complicated by the fact that the organism is homothallic.

SUMMARY

1. The statement is made that *Aspergillus nidulans* produces penicillin in both surface and submerged fermentations.

2. Evidence for this statement includes: *a,* inactivation by penicillinase; *b,* method of extraction; *c,* inactivation by hydroxylamine; *d,* inactivation by copper sulphate; *e,* instability at low and high pH; *f,* solubility in various organic solvents; *g,* antibacterial activity.

3. Of the twenty carbon sources investigated, the whole ground grains supported the best yields. Exclusive of the whole ground grains, lactose and galactose were the best carbon sources. The maximum yield and the time the peak was reached depended upon the concentration of carbon and the amount of aeration. The effect of concentration and aeration varies with the carbon source.

4. No substitute as good as corn steep was found. Soy bean meal was the best of the substitutes among the substances investigated. Corn steep at a 4 per cent concentration, when used with a 2 per cent concentration of lactose, supported the highest yields.

5. The chemical changes occurring during fermentation were studied.

6. The highest yield obtained was equivalent to 20 u/ml. of penicillin.

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PENICILLIN PRODUCTION BY THE ASPERGILLUS NIDULANS GROUP

Eugene L. Dulaney[1]

A number of species of *Penicillium* and *Aspergillus* have been shown to produce penicillin or penicillin-like substances. Within the genus *Penicillium*, the ability to produce penicillin appears to be characteristic of the *P. notatum-chrysogenum* group or of closely related species (**12**). Thus far, among the *Aspergilli,* this ability appears to be more widely distributed except possibly for the *A. flavus-oryzae* group (**12**). While the substances produced by these species of *Aspergillus* have been given various names, they appear on the basis of their biological characteristics to be closely related to penicillin. The substance produced by *A. flavus,* which has been called flavicin (**2**) or flavicidin (**8**) (**9**), has been chemically characterized, provisionally, as an F type penicillin, in which the double bond is in the $\Delta 3$ position (**6**). Gigantic acid, a metabolic product of *A. giganteus* (**10**), is dihydro-F penicillin (**11**). In addition *A. flavus* appears to produce small amounts of penicillin G (**6**). *A. oryzae* (**5**) (**14**) and *A. parasiticus* (**3**), two species closely related to *A. flavus,* also produce substances closely related to penicillin. White (**15**) and Foster and Karow (**5**) list *A. flavipes* as being capable of producing penicillin and the latter workers also report a strain of *A. niger* (**5**) as producing a similar substance. In addition the metabolism solutions of two members of the *A. nidulans* group, *i.e. A. nidulans* (**1**) (**4**) (**5**) and *A. caespitosus* (**1**), contain penicillin-like substances.

With the exception of five strains, all of the cultures used in this survey were members of the Merck culture collection. One strain of *Aspergillus nidulans* was isolated from compost and two strains each of *A. variecolor* and *A. caespitosus* were obtained from K. B. Raper. The nomenclature employed follows that in a *Manual of the Aspergilli* by Thom and Raper.

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Cultures grown on honey peptone agar slants were used as inoculum. Ten milliliters of sterile distilled water were added to seven day old slants, a spore suspension prepared, and one ml. of this suspension added to each flask. The production medium contained the following ingredients in grams per liter of distilled water: corn steep 30 grams, lactose 20 grams, and calcium carbonate 10 grams. The medium was dispensed in 50 ml. amounts in 250 ml. Erlenmeyer flasks which after sterilization and inoculation were placed on a rotary type shaker moving at 175 r.p.m. The incubation temperature was 24° C. The flasks were sampled at three and six days and the metabolism solutions were assayed against *Staphylococcus aureus* by the cylinder plate method. The results are presented in Table 1.

TABLE 1

SURVEY OF 45 STRAINS OF THE ASPERGILLUS NIDULANS GROUP

Species	No. of Strains Tested	No. of Strains Active
Aspergillus nidulans	19	16
A. quadrilineatus	3	3
A. rugulosus	8	3
A. unguis	7	6
A. variecolor	6	6
A. caespitosus	2	2

It can be seen that a majority of the strains were active. With the exception of the *Aspergillus nidulans* strains, one strain of *A. quadrilineatus* and two strains of *A. rugulosus,* the amount of antibiotic produced was only a fraction of one unit. This low level of activity made further experimentation difficult with most of the strains.

Twenty of the most active strains were selected for further study. These included fifteen species of *Aspergillus nidulans,* two of *A. quadrilineatus* and three of *A. rugulosus.* Four day old metabolism solutions of these strains were subjected to a number of tests in order to determine if the activities were due to penicillin-like substances. The results are given below.

ANTIBACTERIAL ACTIVITY. The metabolism solutions were active against *Staphylococcus aureus* but inactive against *Escherichia coli* and penicillin-resistant *S. aureus.* In addition the antibacterial

spectrum of one strain has been compared to that of *Penicillium notatum* 832 and been found to be quite similar.

INSTABILITY AT LOW AND HIGH pH. Portions of the metabolism solutions were adjusted to pH 2.0–2.5 with 1 N HCl and other portions adjusted to pH 10.0–11.0 with 1 N NaOH. After incubation for four hours at 28° C. they were readjusted to approximate neutrality and assayed. In each case the antibiotic activity had either completely disappeared or was greatly diminished.

INACTIVATION BY HYDROXYLAMINE. Hydroxylamine completely inactivated the metabolism solutions after incubation at 28° C. for four hours.

INACTIVATION BY COPPER SULPHATE. Copper sulphate, at a level which did not inhibit *Staphylococcus aureus,* completely inactivated the metabolism solutions after incubation for four hours at 28° C.

ENZYMATIC INACTIVATION. An enzyme preparation which inactivated penicillin, completely inactivated these metabolism solutions after incubation at 37° C. for three hours.

In addition to the above, one strain of *Aspergillus nidulans* has been studied more intensively. The results of this work will be reported at a later date.

Preliminary results indicated that the highest levels of activity were being obtained from strains of *Aspergillus nidulans.* Five of the most active strains were selected for a comparative test on three media: namely, the corn steep (C. S.) medium as given above, the corn steep medium supplemented with a phenyl acetyl derivative at 0.1 per cent level (**7**), and the synthetic medium of Stone and Farrell (**13**). The results are presented in Table 2.

It is quite obvious that use of the supplemented corn steep medium resulted in yields significantly better than those produced on the unsupplemented corn steep medium. In addition strains MF 116 and MF 118 produced yields on the synthetic medium which were significantly higher than those produced on the unsupplemented corn steep medium. The synthetic and unsupplemented corn steep media served equally well for production by MF 119, but the unsupplemented corn steep was slightly superior for strains MF 126 and MF 572.

TABLE 2

COMPARATIVE YIELDS OF FIVE SELECTED STRAINS OF ASPERGILLUS NIDULANS

Strain	Medium	Maximum Yield *S. aureus* Units	Day of Maximum
MF 116	corn steep	11.0	4
	C. S. supplemented	21.0	5
	synthetic	18.0	4
MF 118	corn steep	9.7	4
	C. S. supplemented	21.0	5
	synthetic	14.0	4
MF 119	corn steep	17.0	5
	C. S. supplemented	50.0	5
	synthetic	17.0	5
MF 126	corn steep	15.5	4
	C. S. supplemented	34.0	5
	synthetic	12.0	5
MF 572	corn steep	14.0	5
	C. S. supplemented	21.0	5
	synthetic	11.0	5

The strains used in this survey were from widely different sources. Within the species, the number of strains investigated, with the exception of *Aspergillus nidulans,* was not great, but from the results it would appear that metabolism solutions of species of the *Aspergillus nidulans* group characteristically contain antibiotic factors, even if in small amounts. In addition, results indicate that these factors are closely related to penicillin. Strains of *A. unguis, A. variecolor,* and *A. caespitosus,* while showing activity, were omitted from the later tests because the activity was at such a low level. The latter species, however, has been shown to produce penicillin (1).

It is not clear whether the antibiotic activity of these strains is due to one or more of the now known penicillins, to one or more unknown penicillins, or to a mixture of known and unknown penicillins. The penicillins produced would probably vary with the strain and constituents of the medium. In addition factors other than penicillins may be produced.

While the relatively low levels of activity would seem to eliminate these organisms from commercial consideration, the chemical nature of the active factor(s) should prove interesting.

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THE GROWTH OF FUNGI ON ASPHALT-TREATED PAPER

W. D. Gray and G. W. Martin [1]

(with 7 figures)

The use of asphalt-coated paper to protect the contents of boxes containing materials which might be injured by moisture or molds is very extensive. There seems to be a wide-spread impression that asphalt is itself a fungicide. In view of the very great variability in the substances marketed as asphalt it is quite possible that some asphalts do incorporate materials which are fungicidal, but the studies here reported demonstrate that this cannot be taken for granted.

Several asphalt-treated L-2 Type Case Liners were sent to the Biological Laboratory of the Jeffersonville Quartermaster Depot for study. These were variously spotted (figs. 1 and 2) with what were obviously fungous growths. Isolations made from these spots yielded nineteen different fungi. These were identified at least as to genus and were assigned culture numbers as follows:

Penicillium (*fasciculata* group): J688; J689
Penicillium (*asymmetrica-velutina* group): J677
Penicillium (*biverticillata-symmetrica* group): J697; J699
Penicillium (*lanata typica* group): J698
Aspergillus (*flavus-oryzae* group): J674
Aspergillus (*versicolor* group): J686; J687; J695
Aspergillus (*fumigatus* group): J675
Aspergillus spp.: J694; J673; J696; J589
Chaetomium sp.: J647
Sepedonium sp.: J645
Trichoderma sp.: J646
Chaetomium globosum: no number assigned

Since the isolation work had shown that the L-2 Type Case Liner material involved would support the growth of a number of fungi, it seemed advisable to obtain materials of this nature from various firms and determine if they would also support fungus

[1] Formerly of Jeffersonville Quartermaster Depot. Publication authorized by Office of Quartermaster General.

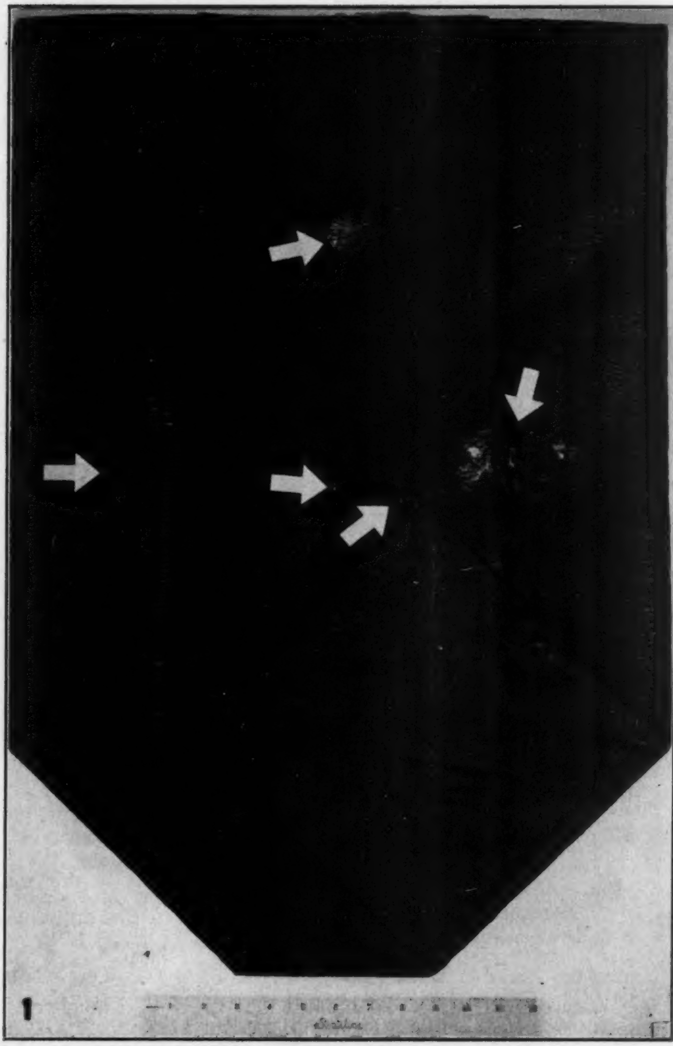

FIG. 1. L-2 Type Case Liner showing fungous spotting.

growth. Accordingly, finished case liner materials, kraft paper, and both infusion and laminating asphalt were obtained from three different sources in order that these materials might be tested to determine if they were subject to fungus attack. In addition to these materials, creped kraft paper treated with Dowicide G, ranging in concentration from 0.7 to 1.6 per cent, was also obtained and subjected to test.

Experimental procedure. The method generally employed for testing finished liner material, asphalt-infused paper, laminated paper, and plain or creped kraft paper was to cut test squares (2 inches × 2 inches), sterilize them by autoclaving, and then transfer each test square to a petri dish containing sterile nutrient salt agar. Inoculations were made by placing a small drop (1–2 mm. diameter) of sterile distilled water in the center of each square and introducing spores of a single species of fungus into the drop; the inoculated plates were then incubated in a room in which the temperature and humidity were controlled at 85° F. and 85 per cent R.H.

Samples of asphalt were tested as follows: small spheres (*ca.* 5 mm. diameter) were placed in the centers of 1 × 3 inch microscope slides; each slide was then heated over a low flame and was rocked gently until an area of approximately one square inch was covered with asphalt. The slides with asphalt were then sterilized by autoclaving and placed on the surface of nutrient salt agar in petri dishes. Inoculations were made by introducing spores into a small drop of sterile distilled water placed in the center of the asphalt film. In one instance the sample of asphalt was not in the solid state, so it was simply autoclaved, and small amounts were placed on the surface of sterile nutrient agar in petri dishes and allowed to spread; inoculations were then made as described above.

Results. Firm No. 1 supplied samples of its finished asphalt-treated paper which consisted of an asphalt-saturated sheet of smooth kraft paper laminated to a sheet of unsaturated smooth kraft paper. This firm also supplied samples of saturating asphalt and laminating asphalt. Samples of liner material and asphalt were inoculated with several organisms isolated from the original defective liners, incubated, and observed at five- and ten-day intervals. Results obtained with these materials are presented in Table I. A

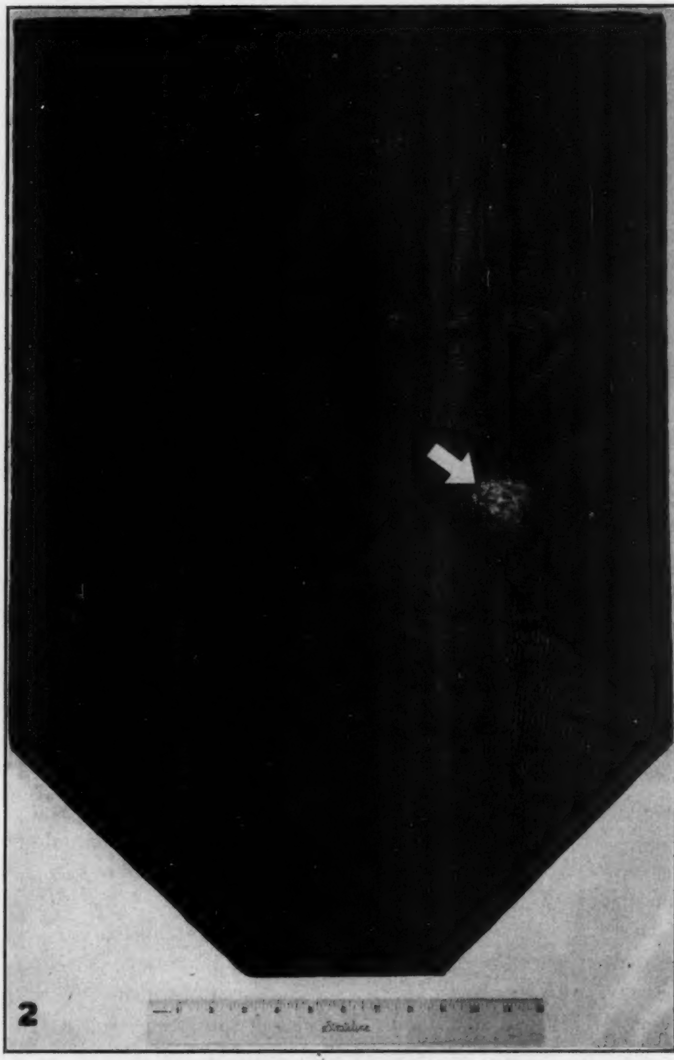

FIG. 2. Reverse side of the liner shown in first figure.

TABLE I

GROWTH OF FUNGI ON MATERIALS SUPPLIED BY FIRM NO. 1

Organism	Culture No.	Growth*					
		Finished Product		Saturating Asphalt		Laminating Asphalt	
		5-day	10-day	5-day	10-day	5-day	10-day
Penicillium	J688	x	xx	x	x	–	–
Aspergillus	J686	–	x	x	x	–	–
Aspergillus	J675	x	xxx	x	x	–	x
Aspergillus	J674	x	xxxx	–	x	–	xx
Penicillium	J689	x	xx	x	x	–	–
Chaetomium	J647	x	xxxx	x	x	x	x
C. globosum	—			x	x	x	x
Sepedonium	J645	–	xx	–	–	x	x

* In this and all subsequent tables the following designation is used:

–	no growth
x	up to ¼ of total area covered
xx	¼ to ½ of total area covered
xxx	½ to ¾ of total area covered
xxxx	over ¾ of total area covered

photograph of a test square of the finished liner material, inoculated with *Chaetomium* sp. (J647), is presented in Figure 3. This photograph was taken nine days after inoculation.

Firm No. 2 supplied two types of material: (1) the finished product, which was laminated, two-ply, asphalt-coated, creped kraft paper, and (2) two sheets laminated with asphalt but not coated or creped. Results obtained with these materials are listed in Table II.

TABLE II

GROWTH OF FUNGI ON MATERIALS SUPPLIED BY FIRM NO. 2

Organism	Culture No.	Growth			
		Finished Product		Laminated Only	
		5-day	10-day	5-day	10-day
Penicillium	J688	–	–	–	x
Aspergillus	J686	x	x	x	x
Aspergillus	J675	x	xxxx	x	xxxx
Aspergillus	J674	xx	xxxx	xxx	xxxx
Penicillium	J689	–	x	x	x
Chaetomium	J647	x	xxxx	–	xx
C. globosum	—	x	xxxx	x	xxx
Sepedonium	J645	x	x	x	x

Firm No. 3 supplied the following materials: (1) finished product (two-ply, asphalt-treated, laminated, both sheets creped), (2) same as the preceding except that the uninfused sheet was a smooth kraft paper, (3) single, creped, asphalt-infused sheet, (4) smooth, untreated kraft paper, (5) creped, untreated kraft paper, (6) infusion asphalt, and (7) laminating asphalt. Results obtained from tests conducted with these materials are presented in Tables III and IV; photographs made nine days after inoculation are shown in Figures 4, 5, and 6.

TABLE III

GROWTH OF FUNGI ON PAPER AND PAPER-PRODUCTS SUPPLIED BY FIRM NO. 3

(Observations made ten days after inoculation)

Organism	Culture No.	Growth				
		Finished (Plain-back)	Finished (Creped back)	Infused sheet	Smooth Kraft	Creped Kraft
Penicillium	J688	xx	x	x	x	xx
Aspergillus	J686	x	x	x	x	x
Aspergillus	J675	xxxx	xxxx	xxxx	xxxx	xxxx
Aspergillus	J674	xxxx	xxx	xxxx	xxxx	xxxx
Penicillium	J689	xx	x	xx	xxx	xx
Chaetomium	J647	xxxx	x	xxxx	xxxx	xxxx
C. globosum	—	xxxx	x	xxxx	x	xxxx
Sepedonium	J645	x	x	x	x	x

TABLE IV

GROWTH OF FUNGI ON ASPHALTS SUPPLIED BY FIRM NO. 3

Organism	Culture No.	Laminating Asphalt		Infusion Asphalt	
		5-day	10-day	5-day	10-day
Penicillium	J688	–	x	–	x
Aspergillus	J686	–	x	–	x
Aspergillus	J675	–	x	x	x
Aspergillus	J674	–	–	–	x
Penicillium	J689	x	x	–	–
Chaetomium	J647	–	–	x	x
C. globosum	—	x	x	x	x
Sepedonium	J645	–	–	x	x

From the results presented in Tables I–IV, it is obvious that all of the constituent materials of L-2 Type Case Liners which were tested are capable of sustaining the growth of a number of fungi.

Whether or not the paper is appreciably damaged by all of the fungi which have been shown to be capable of growing on it cannot be accurately determined by observations of the type described above; hence, a number of the organisms employed in these studies were

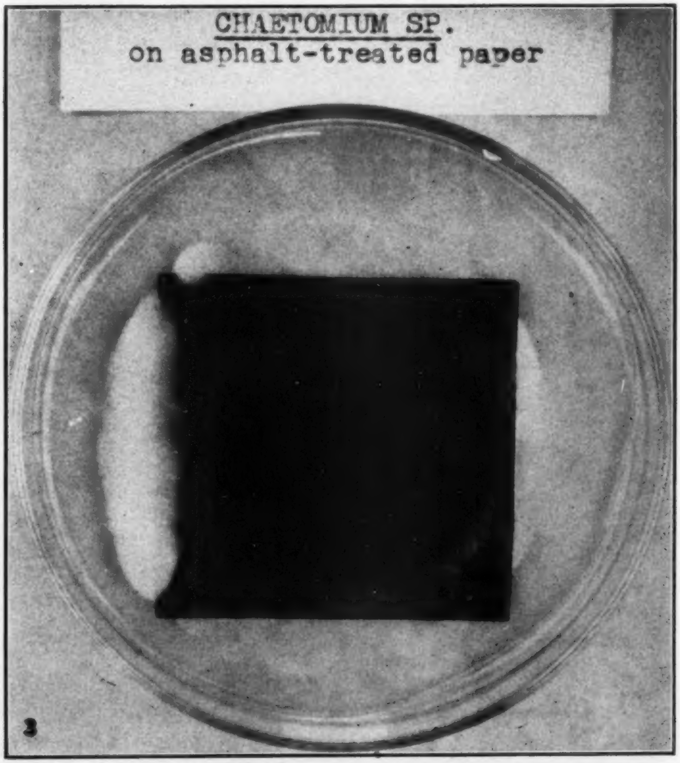

FIG. 3. Growth of *Chaetomium* sp. (J647) on finished liner material. Photographed nine days after inoculation.

examined from the standpoint of their cellulose-destroying abilities. This was determined by measuring changes in tensile strength of gray duck inoculated with various of these organisms. Strips of 12.29 oz. gray duck ($1\frac{1}{2} \times 5\frac{1}{2}$ inches, warp threads in the long dimension) were ravelled to a width of one inch; each strip was

then placed in a 1 × 8 inch test tube containing 10 ml. of nutrient salt solution and sterilized by autoclaving. Each strip was inoculated by streaking spores in a single vertical line about one inch long near the middle of the strip; ten replicate strips were inoculated with each fungus tested. After seven days incubation at 85° F. and 85 per cent R.H. the strips were washed, dried, and then conditioned for 24 hours at 72° F. and 65 per cent R.H. prior to breaking. The strips were broken on a 0–250 lbs. Scott testing machine. Results of tensile strength determinations are listed in Table V.

TABLE V

CHANGE IN TENSILE STRENGTH OF 12.29 OZ. GRAY DUCK INOCULATED WITH FUNGI WHICH HAD BEEN ISOLATED FROM DEFECTIVE L-2 TYPE CASE LINERS

Organism	Culture No.	Average Tensile Strength	Per Cent Change in Tensile Strength
Penicillium	J688	171.2	3.4% loss
Aspergillus	J686	182.0	2.5% gain
Aspergillus	J675	110.3	37.8% loss
Aspergillus	J674	142.9	19.4% loss
Penicillium	J689	162.8	8.1% loss
Aspergillus	J687	166.5	6.1% loss
Chaetomium	J647	61.3	65.4% loss
Sepedonium	J645	133.7	24.6% loss
Trichoderma	J646	62.1	36.8% loss
Uninoculated controls		177.2	

The results in Table V show that many of the fungi which can grow on asphalt-treated paper are capable of destroying cellulose and hence can damage the paper very appreciably. It should be noted that the ten strips inoculated with *J686* showed an average gain in tensile strength of 2.5 per cent. No especial significance should be attached to this fact other than that this fungus is incapable of attacking cellulose under the conditions of the test. The slight apparent gain in tensile strength was probably due either to variations in the tensile strength of the original cloth or to a slight shrinkage and tightening of the fibers during the course of the test.

Since all of the materials tested were shown to be susceptible to attack by various fungi, paper into which a fungicide was incorporated was obtained and subjected to test. Four lots of such paper were received; these contained 0.7, 0.9, 1.2, and 1.5 per

FIG. 4. Growth of *Penicillium* sp. (J674) on materials supplied by Firm No. 3.

cent Dowicide G, respectively. In the preliminary experiment both sterilized and unsterilized test squares of treated paper were inoculated with nine test organisms. Test squares of sterilized, untreated, creped kraft paper supplied by Firm No. 3 were used as controls. After ten days incubation the test squares were examined, and the controls were found to have supported growth of all the fungi tested. There was no growth on either the sterilized or non-sterilized samples containing Dowicide G, as is illustrated by Figure 7.

Test squares of fungicide-containing papers were next leached in running water for 24 hours; they were then dried, sterilized and inoculated. Three test squares from each lot were then inoculated with *Chaetomium sp.* (J647), three with *C. globosum*, and six with enriched soil suspension. After ten days incubation only one test square in twelve of the paper which contained 1.5 per cent inhibitor prior to leaching supported fungus growth. This was true of the paper containing 1.2 per cent inhibitor, whereas the papers originally containing 0.7 and 0.9 per cent inhibitor supported fungus growth in over one-half of the test plates. When the various papers were subjected to leaching for a one hundred and twenty hour period, enough inhibitor was removed to permit fungus growth on all test squares. These experiments demonstrate that whereas Dowicide G may protect paper if present in a concentration of 0.7 per cent or higher, it may be leached from the paper rather readily. The fact that this inhibitor is not fast to leaching would probably be unimportant if the paper containing it was infused or coated with asphalt.

In spite of the fact that kraft paper can be treated with Dowicide G and thus rendered mildew-resistant, the question arises as to whether or not paper containing an inhibitor can support fungus growth when infused or coated with asphalt not containing an inhibitor. Accordingly, materials were prepared in order to answer this question. Both paper containing Dowicide G and untreated paper were infused with plain asphalt and asphalt containing 3 per cent pentachlorophenol. One inch square test strips were used; these were dipped in a solution of 5 gms. of asphalt dissolved in 25 ml. of ethyl ether. After dipping, the test squares were dried in a vacuum oven for four hours. When inhibitor was

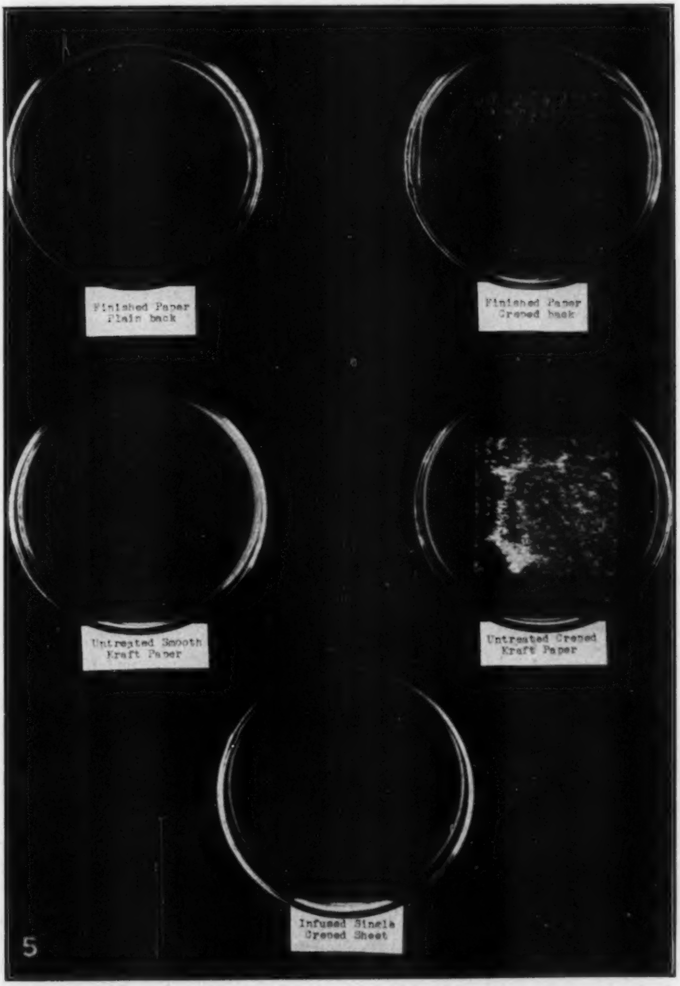

FIG. 5. Growth of *Aspergillus* sp. (J675) on materials supplied by Firm No. 3.

incorporated in the asphalt, 0.15 gm. of pentachlorophenol was dissolved in the ether-asphalt solution. The following types of samples were prepared:

1. Treated paper infused with untreated asphalt.
2. Untreated paper infused with treated asphalt.
3. Untreated paper infused with untreated asphalt and laminated to treated paper with the same type of asphalt.
4. Treated paper infused with treated asphalt.

Sterilized and unsterilized samples of the above materials were inoculated and observed after a ten day incubation period. Since no differences appeared between sterilized and unsterilized samples, only the results obtained with unsterilized samples are presented in Table VI.

TABLE VI

GROWTH OF FUNGI ON VARIOUS COMBINATIONS OF TREATED AND UNTREATED PAPER WITH TREATED AND UNTREATED ASPHALTS

Organism	Culture No.	Paper: 1.6% Dowicide G; Asphalt: untreated	Paper: untreated; Asphalt: 3% pentachlorophenol	Untreated paper infused and laminated to treated paper with untreated asphalt.	Paper: 1.6% Dowicide G; Asphalt: 3% pentachlorophenol
Penicillium	J688	x	–		–
Aspergillus	J686	xxx	–		–
Aspergillus	J675	xx	–		–
Aspergillus	J684	xx	–	xx	–
Penicillium	J689	xx	–	x	–
Chaetomium	J647	x	–	x	–
Sepedonium	J645	x	–	x	–
Trichoderma	J646	xx	x	xxx	–

DISCUSSION AND CONCLUSIONS. On the basis of the above studies it may be concluded that a large number of fungi are able to grow on asphalt-treated paper or its component materials. Of the various materials tested, none was found that is naturally resistant to fungus attack. Some of the fungi involved are capable of attacking cellulose (as evidenced by loss of tensile strength by 12.29 oz. gray duck); others appear to be merely surface growers. While members of the genus *Aspergillus* are ordinarily considered surface growers, it should be noted that one species of *Aspergillus* (J675) was able to reduce the tensile strength of gray duck 37.8 per cent in one week.

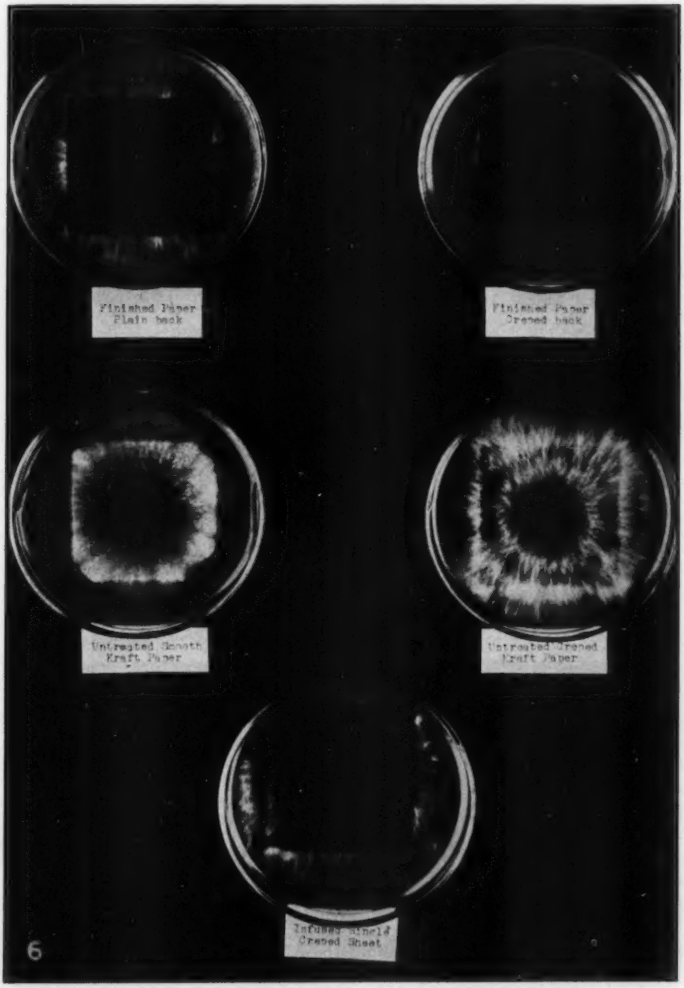

FIG. 6. Growth of *Chaetomium* sp. (J647) on materials supplied by Firm No. 3.

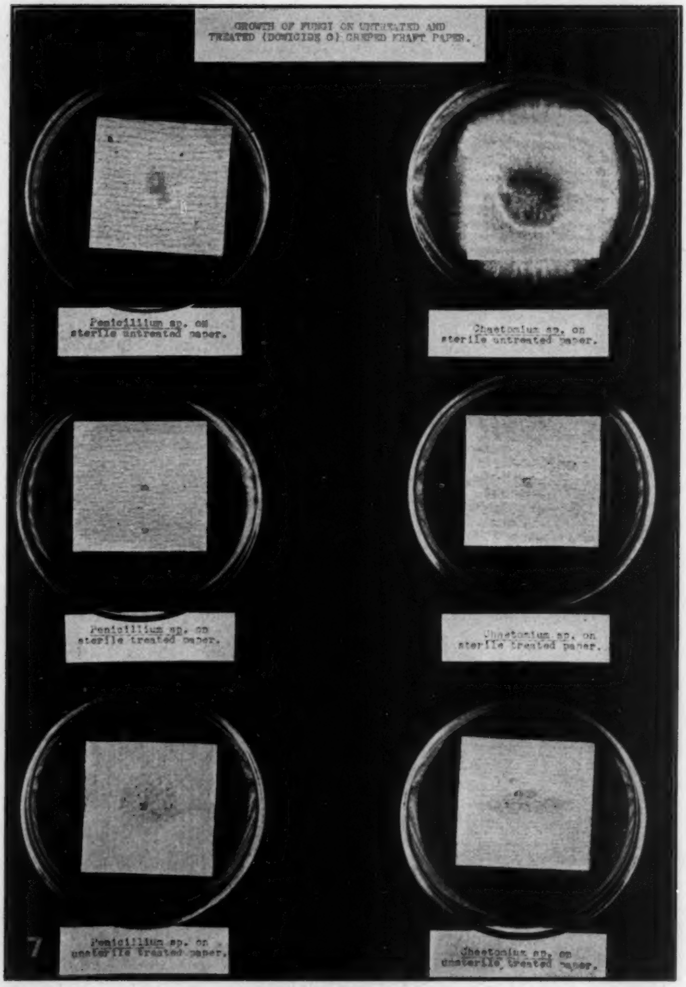

FIG. 7. Growth of *Penicillium* sp. (J674) and *Chaetomium* sp. (J647) on creped kraft paper, with and without Dowicide G.

Treatment of kraft paper with Dowicide G resulted in a product which effectively resisted fungus attack, even when incorporated into the paper in amounts as low as 0.7 per cent. Leaching of paper so treated resulted in a paper which supported some fungus growth; however, the fact that this inhibitor can be leached out should not be a deterrent factor in its use, since it is doubtful that much, if any, could be leached out when the paper is coated with asphalt.

Asphalt-coated case liner material prepared with treated paper and untreated asphalt was shown to be able to support fungus growth, so for complete mildew resistance it is necessary to incorporate an inhibitor in the asphalt as well as in the paper. Paper containing 1.6 per cent Dowicide G, infused with asphalt containing 3.0 per cent pentachlorophenol, was found to be completely mildew-resistant. Untreated paper infused with treated asphalt was found to be resistant to all of the test fungi except a species of *Trichoderma* (J646), so a reasonable degree of mildew resistance can be attained by adding inhibitor to the asphalt only. However, this method of treatment cannot be recommended unless the paper is very thoroughly infused with treated asphalt.

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SPECIES OF THE GENERA DOASSANSIA, DOASSANSIOPSIS, AND BURRILLIA IN INDIA

M. J. Thirumalachar

(with 9 figures)

Species of the genera *Doassansia* Cornu, *Doassansiopsis* (Setch.) Diet. and *Burrillia* Setchell are of extreme interest to workers on smuts on account of their rarity and the interesting types of life-cycles in many of the species. The rarity of these smuts is often due to the very obscure symptoms they produce on the host, which might range from an almost inconspicuous paling of the infected portions to definite discolored spots. In very few cases there is the formation of tumor-like excrescences on the host. In India, three species of *Doassansia* have been known by the determinations of Sydow and Butler. Another species, *Doassansia Hygrophilae* Thirumalachar, was added by the writer (**1946**). It is based on a collection of a leaf smut made near Nandi, Mysore, on *Hygrophila* sp.

No species of *Burrillia* was hitherto known to occur in India, and a first report of an undescribed species of *Burrillia,* viz. *B. Narasimhanii,* was made recently by Thirumalachar and Mundkur (**1946**) on *Alisma reniformis* G. Don. This has been followed up by the finding of another species of *Burrillia* on *Monochoria vaginalis* Presl. by the writer and it appears to be undescribed. The life-cycle of the smut has been worked out in some detail, and in this paper an account of it is presented. Some of the observations made on the other species of *Doassansia* and *Burrillia* are also included.

The genus *Doassansia* was founded by Cornu in 1883, with *D. Alismatis* (Nees) Cornu as the type. The sori are embedded in various parts of the host and are composed of hyaline to pale-yellow spore balls which are permanently embedded. There is usually a distinct layer of cortex surrounding the spore ball.

Setchell (**1892**), who studied the numerous species of *Doassansia* in considerable detail, recognized three subgenera of which *Doassansiopsis* was stipulated for those species in which the spore balls, instead of being filled by fertile spores, are made up of sterile pseudoparenchymatous cells. The fertile region is confined to a single outermost layer, and the entire spore ball is enclosed within a sterile cortex or hyphal sheath. The subgenus was raised to generic rank by Dietel (**1897**), but the genus is credited to Setchell by Liro (**1938**) and Ciferri (**1938**).

Doassansia Alismatis (Nees) Cornu. Ann. Nat. Sci. (Bot.) vi, xv, 269–296, 1883; Mundkur, Trans. Brit. Myc. Soc. xxiv, 312–336, 1940. On the leaves of *Alisma plantago* L. Achibal, Kashmir, 2–9–1908, leg. E. J. Butler.

Doassansia Hygrophilae Thirumalachar. Lloydia, **9**: 24–30, 1946. On the leaves of *Hygrophila* sp.

Doassansia Hygrophilae is a leaf smut on *Hygrophila,* a member of the Acanthaceae. The spore balls are permanently embedded within the leaf tissue, presenting a punctate appearance on the surface. The spore balls possess a distinct sterile cortex and conform to the *Eudoassansia* type of Setchell. The mycelium and the young spores possess two nuclei, the mature spores having a single fusion nucleus. The spores have been germinated and have been found to develop a promycelium bearing a whorl of 6 to 7 sporidia which conjugate in pairs after they get loosened from the promycelium. The diploid mycelium formed after the conjugation of the sporidia has been found to develop secondary conidia in some cases.

DOASSANSIOPSIS MARTIANOFFIANA (Thum.) Diet. Engler and Prantl's Die Natürlichen Pflanzenfamilien, 1**, 2–24, 1897. On floating leaves of *Potamogeton* sp., in Walur Lake, Kashmir, 17–9–1908, leg. E. J. Butler.

The smut is foliicolous forming circular orange-yellow spots, which gradually turn brownish-red. The spore balls are embedded within the mesophyll and in the early stages of development they appear to be applanate and compressed, surrounded by a thick felt of hyphal sheath. As the spore balls mature, a single fertile layer of spores is seen, surrounding a central core of sterile pseudoparenchymatous mass of cells. The sterile hyphal sheath

also gradually becomes less conspicuous and later becomes evanescent. In mature spore ball its presence is indicated only by a delicate layer of hyphal cells which could be made out only with some difficulty. On the other hand, in the immature spore balls it is very conspicuously noticed and is composed of several layers. Clinton (**1906**) refers to this layer as merely a sterile cortex. Liro (**1938**) refers to these enveloping cells which are stated to measure 5 to 8 μ in diam. Dietel (**1928**) on the other hand differentiated between "Rindenzellen" which mean the cortical cells as distinct from the interlacing mass of hyphae surrounding the spore balls and states that the cortical layers are definitely absent in *D. Martianoffiana*. Ciferri (**1938**) also describes the outer sheath as being made up of lax interlaced hyphae and it seems desirable to identify such a layer as distinctly separate from the compact cortical layers found in many species of *Doassansia*.

Doassansiopsis Nymphaeae (Syd.) comb. nov. Petiolicolous, forming conspicuous tumor-like cushions, 5–16 mm. long. Spore balls compactly grouped within spaces formed in the tissue, densely clustered; angularly globoid to elliptical, 170–250 μ in diam., surrounded by a delicate layer of hyphae; spores ellipsoidal to prismatic, forming a single outermost layer, 12–16 × 7–11 μ, pale cinnamon brown, smooth, surrounding a central mass of sterile pseudoparenchymatous cells which are polygonal, thin-walled, measuring 10–25 μ.

On the leaves of *Nymphaea stellata* Willd., near Bassein, Bombay Presidency, 18–2–1912, leg. H. M. Chibber.

The smut was first collected by Chibber at Nirmal, Bassein and described by Sydow (**1912**) as *Doassansia Nymphaeae* Syd. and has so far not been reported from any other locality. A small fragment of the type became available for study, and some of the observations made by the writer proved the smut to be a species of *Doassansiopsis*. The smut produces hemispherical cushion-shaped galls on the petioles (FIG. 1). The spore balls are compactly grouped in clusters in lacunae formed within the hypertrophied tissue of the host, the margin of the cavity being lined with a felt of hyphae (FIG. 2). The spore balls are permanently embedded and do not show any tendency to get dislodged from their place. In carefully prepared sections of the spore balls, it was observed

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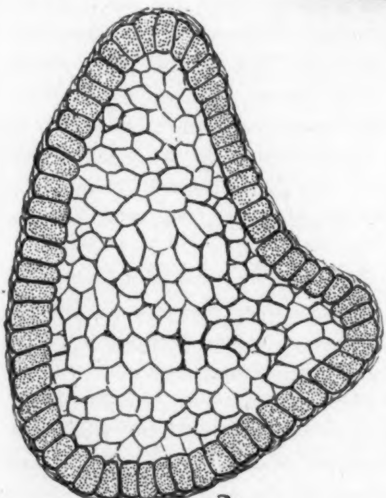

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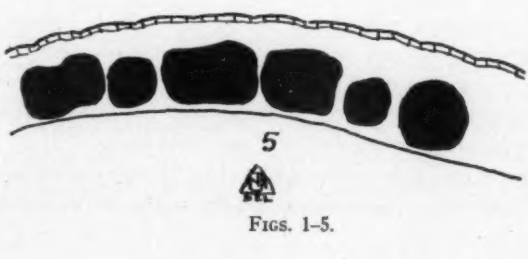

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FIGS. 1–5.

that the entire central mass of cells is composed of sterile pseudoparenchymatous cells. These do not possess any contents, and on crushing do not get separated as in the case of fertile spores. They are thin-walled, and of varying sizes, measuring 10–25 μ in diam. The fertile spores are grouped in a single layer and are ellipsoid to prismatic in shape. The cells are rich in contents and not vacuolate as in the sterile pseudoparenchymatous cells of the center. The spore ball on careful examination reveals a delicate layer of hyphae surrounding it.

All the above-mentioned characters clearly indicate that the smut is a species of *Doassansiopsis*. When an entire spore ball is examined under the microscope, the fertile spores in the margin of the field give the appearance of a sterile cortex and one is apt to mistake it for a species of *Doassansia*. Sydow, who considered the smut to be a species of *Doassansia*, describes a cortical layer which in fact refers to the fertile layer of spores. The spores are stated to be 8–11 μ in diameter and this corresponds with the breadth of the spores (7–11 μ) which in a polar view of the spore ball appear as rounded structures (FIG. 2). The pseudoparenchymatous cells in the center are actually much larger in size, measuring 10–25 μ in diam.

BURRILLIA NARASIMHANII Thirumalachar and Mundkur. *Papers in the Imp. Mycological Institute, Kew, England* (in press). On the leaves of *Alisma reniformis* G. Don., Donayakanapalya, Belur, Mysore, 26–9–1945, leg. M. J. Thirumalachar.

The genus *Burrillia* Setchell was established by Setchell (**1891**) for the accommodation of a smut on *Sagittaria variabilis* which was placed by Dietel as *Doassansiopsis pustulata* Diet. The genus is distinguished from *Doassansia* by the absence of any sterile cortex, but with or without the association of sterile parenchymatous cells in the spore ball. In the former case the spore balls would be associated with a few sterile cells which become distributed irregularly, and in the latter the entire spore ball is composed of fertile spores. With respect to the shape, structure and germination of the spores as far as they are known, there is close resemblance between *Burrillia* and *Doassansia*.

The smut causes pale yellow spots which in many cases are not distinctly observed. Sections through the region of the discolored

spot reveal the spore balls within the mesophyll. The spores are not firmly united to form a compact ball, but are packed in crustose layers and thus resemble the spore balls of *Burrillia anomala* Crowell, described on a member of the Typhaceae in Canada (Crowell, **1940**). The spores of *Burrillia Narasimhanii* have been germinated and it has been found that the sporidia do not conjugate in pairs after their formation, but begin to form secondary sporidia acrogenously.

Burrillia Ajrekari Thirumalachar sp. nov. Sori in foliis efformantes maculas irregulares ad circulares, discoloratas, 3–15 mm. magnitud.; hae vero maculae sunt dispersae vel coalescentes, luteo-albae in inferiore facie, pallide violaceae in superiore facie. Sporarum globi infixi in mesophyllo, 120–197 × 114–306 μ, subgloboso ad rhomboidales, coalescentes inter se cum vicini sunt; sporae sparse distributae in sporarum globo, inter se unitae cellulis parenchymaticis tenuiter parietatis, pallide cinnamomo luteae, globosae ad sphaericas, tenuibus parietibus praeditae, magnitudinis 10–15 μ (medio 9.4). Sporae germinant in maturitate per 1–2-septatum promycelium quod producit 6-sporidia verticillata; haec vero statim bina conjugantur.

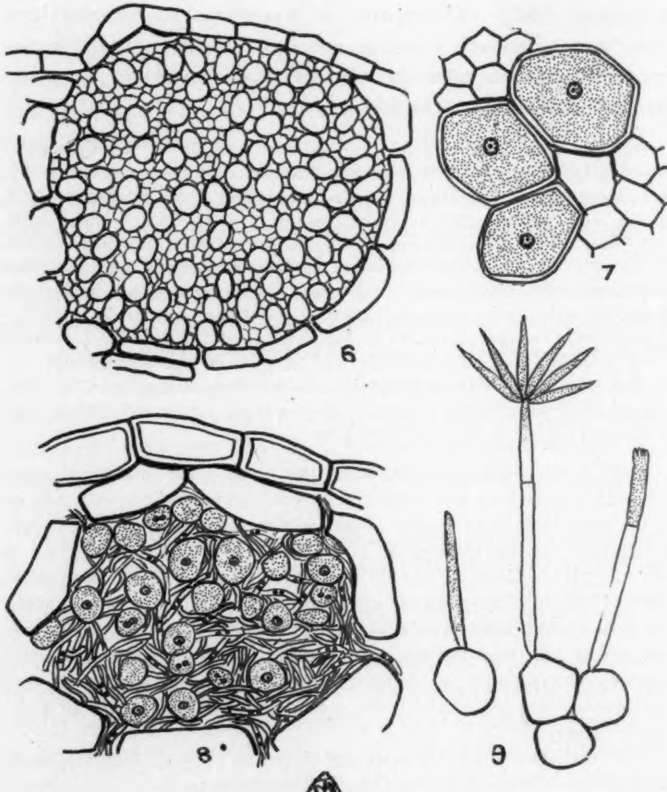
Hab. in foliis *Monochoriae vaginalis,* Bannerghatta, Bangalore, 23–6–1946, leg. M. J. Thirumalachar. Typus positus in Herb. Crypt. Ind. Orient. New Delhi, et in Herb. I. M. I., Kew, Anglia.

Sori in the leaves forming irregular to circular discolored spots of 3–15 mm. which are scattered or coalescent, yellowish-white on the lower surface and pale violet on the upper side. Spore balls embedded in the mesophyll 120–197 × 114–306 μ, subglobose to rhomboidal, coalescing with one another if in close proximity; spores sparsely distributed within the spore balls, joined together by thin-walled parenchymatous cells, pale cinnamon-yellow, globose to spherical, thin-walled, measuring 10–15 μ (mean 11.7 μ). Spores germinating at maturity by one- to two-septate promycelium bearing a whorl of six sporidia which conjugate in pairs immediately.

On the leaves of *Monochoria vaginalis* Presl., Bannerghatta, Bangalore, 23–6–1946, leg. M. J. Thirumalachar.

A leaf smut on *Monochoria vaginalis* growing in the marshy regions near Bannerghatta, a suburb of Bangalore, proved on a detailed examination to be a species of *Burrillia. Monochoria vaginalis* is a member of the Pontederiaceae, and Ciferri (**1928**) has described *Doassansia Eichorniae* Cif. on *Eichornia crassipes* from Santo Domingo. According to Zundel and Barnhart (**1939**), *D. Eichorniae* is the only smut known parasitizing a member of

the Pontederiaceae. The *Burrillia* collected by the writer would therefore constitute a second smut species to parasitize a member of that family.



FIGS. 6-9.

The infection is very obscure, appearing in early stages by very slightly discolored patches on the lower surface. There is no indication or visible symptom of infection on the upper surface in the early stages. As the spore balls begin to be fully formed, the discoloration on the lower surface becomes more prominent and on the

upper surface a greenish-violet tinge in the infected regions can be observed. The discolored patches on the lower surface measure 3–15 mm. in diam., and appear to coalesce with one another, forming a continuous patch (FIG. 4).

Sections through the leaf in the region of the infection patch reveal the presence of numerous spore balls embedded within the mesophyll tissue. These are mostly placed between the lower epidermis and the palisade layer, and measure 120–197 × 114–306 μ. They are subglobose to rhomboidal in shape and appear to coalesce with one another when in close proximity. The mycelium along the margin of the infection patch spreads and develops young spore balls in the mesophyll tissue, with the result that the mature spore balls are always situated towards the center and the younger ones along the periphery (FIG. 5). The spore balls which cannot be made out macroscopically on a fresh leaf appear in the dried leaf as small papillate pustules on the surface of the infection patch.

For cytological studies, the material was fixed in formalin acetic alcohol and microtome sections of 8 to 10 μ thickness were cut and stained with Newton's iodine gentian violet with light green as counterstain. For germinating the chlamydospores, the leaves bearing the sori were teased on the slides to release the spore balls. These were then germinated and stained by the method suggested by the writer (**1940**).

The mycelium within the host is visible before it gets grouped to form young spore balls. The mycelium leading up to the young spore balls is very fine and delicate, septate and distinctly binucleate. A large plectenchymatic mass is formed, two to four layers beneath the epidermal cells. The cells of the hyphae are closely intertwined with one another and as development proceeds, the zone of the hyphal cells becomes rounded up and possesses densely staining contents. The hyphal cells are binucleate and hence the young spores that are formed also possess the two nuclei (FIG. 8). The spores that are thus produced are very sparsely distrubuted within the spore ball and bound up by the remaining hyphal cells (FIG. 6). In later stages, these connecting hyphal cells also enlarge to some extent in size, lose their protoplasmic cell contents and become transformed into sterile parenchymatous cells. These cells are

smaller than the spores in size and therefore can be clearly differentiated. In the mature spores (FIG. 7) there is a single fusion nucleus. The spores are ovate-ellipsoid to subspherical, pale cinnamon-yellow and measure 10–15 μ in diameter.

The spores were germinated and stained by the method already described. They require three to four days to germinate. The promycelium protrudes out by the rupture of the spore coat and the contents of the cell migrate into the germ tube thus formed. As in some of the species of *Doassansia* and *Burrillia* in which the germination stages are so far known, the promycelium becomes 1–2-septate, the septa separating off the empty portion of the promycelium. A whorl of 6 sporidia is produced at the apex (FIG. 9). The young sporidia are seen in early stages as small papillate projections and when excess of moisture is present, particularly in the case of submerged spores, the sporidia formed taper off into long whip-like structures which are slightly recurved. The further development of these sporidia which readily get detached from the promycelia is unknown. In those cases where the promycelium develops sporidia above the surface of water or without there being excess of moisture there is no tendency for them to elongate into whip-like branches, but instead they remain cylindric to fusoid, broader at the base than at the apex. Soon after their formation the sporidia begin to conjugate in pairs while still attached to the promycelium. The conjugation tubes are produced in the equatorial regions and by their fusion H-shaped pieces are produced. In *Burrillia pustulata* Setchell (**1892**) records a similar type of sporidial conjugation. On the other hand, in *B. Narasimhanii* the sporidia, soon after their formation, develop secondary sporidia acrogenously and have never been observed to conjugate with one another.

The present species of *Burrillia* differs in the structure of the spore balls and the measurements of the spores from any of the species so far known on other hosts, and the name *Burrillia Ajrekari,* in honor of Professor S. L. Ajrekar, is proposed.

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EXPLANATION OF FIGURES

FIG. 1. Petiole of *Nymphaea stellata* with the tumors of *Doassansiopsis Nymphaeae*. Natural size.

FIG. 2. Spore balls clustered within the lacunae of the host. × 100.

FIG. 3. Spore ball of *D. Nymphaeae*. × 400.

FIG. 4. Leaf of *Monochoria vaginalis* with the infection patch. Natural size.

FIG. 5. Showing the position of the spore balls in the section of a leaf. × 80.

FIG. 6. Spore ball of *Burrillia Ajrekari* showing the sparsely distributed fertile spores within the spore ball. × 400.

FIG. 7. Mature spores showing the fusion nucleus. × 1800.

FIG. 8. Stages in the development of the spore ball. × 900.

FIG. 9. Germination of chlamydospores. × 900,

RHIZOPHYDIUM CHITINOPHILUM

Grace Antikajian [1]

(with 20 figures)

In making routine collections of leaf mold, moist soil, and fresh water to be used as sources of chytrids for the mycology course at Columbia University, a new species of *Rhizophydium* was collected in Van Cortlandt Park, New York City, and isolated on strips of purified shrimp chitin.

All attempts to grow it on other substrata such as cellophane, bleached sections of young corn leaves, onion skin, hemp seed, hair, feathers, pollen of several species of angiosperms and of pine were unsuccessful. So far it has grown only on chitin or chitin agar as prepared by Karling (**1945**), and it differs in this respect from other known species of *Rhizophydium*. Morphologically, this species is characterized by large, hyaline, spherical, oval, or pyriform sporangia, unusually coarse and extensive rhizoids, spherical zoospores containing a minute, hyaline, refractive globule and a larger, less refractive, granular body, and by smooth, brown, coarsely granular resting spores of various sizes and shapes. In this latter characteristic it differs specifically from other species of *Rhizophydium* which have been described in the literature. *Rhizophydium Closterii* Karling (**1946**), *R. Amoebae* Karling (*l.c.*), and *R. mycetophagum* Karling (*l.c.*) have brown resting spores like our species but the wall is either warty or the spores include one or more large refractive globules in contrast to the coarsely but evenly granular content of the species from Van Cortlandt Park.

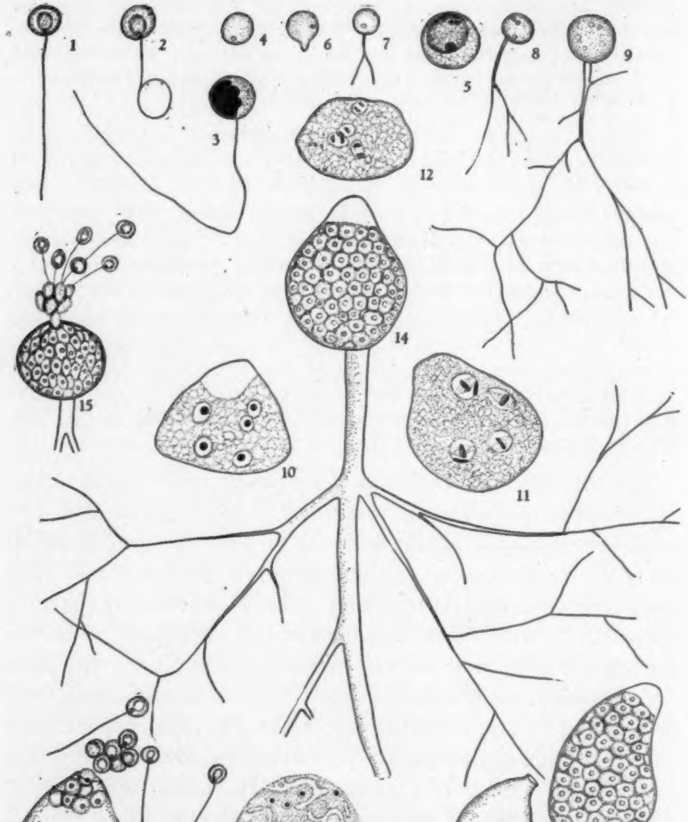
In view of these structural characteristics and differences, this fungus is regarded as a new species and named *Rhizophydium chitinophilum* because of the type of substratum to which it is restricted.

[1] The writer wishes to express her sincere appreciation to Professor J. S. Karling for his helpful advice during the course of this study.

Rhizophydium chitinophilum sp. nov. Fungus saprophyticus; sporangiis sessilibus, hyalinis, laevibus, sphaericis (7–69 μ), aut ovalibus (7–66 × 11–87 μ), aut pyriformibus (8–58 × 11–96 μ); 1–2 papillatis; zoosporis sphaericis (3.6 μ), flagello (21–26 μ) longo; sporis perdurantibus laevibus, fuscis, sphaericis (9–18 μ), aut ovalibus (7–14 × 9–17 μ), aut pyriformibus (7–11 × 11–14 μ), aut leviter irregularibus; prosporangiis germinantibus zoosporangia membranacea superficialia gerentibus.

Sporangia sessile, hyaline, smooth, spherical (7–69 μ), subspherical (15–66 × 17–74 μ), oval (7–66 × 11–87 μ), or pyriform (8–58 × 11–96 μ); neck of sporangium (4–17 × 11–28 μ); exit papillae one or two, low and broad, usually apical, filled with hyaline matrix (4–13 μ) high and (7–21 μ) wide. Zoospores spherical, approximately 3.6 μ with a minute refractive globule less than 0.7 μ and a large somewhat refractive, granular body about 2.2 μ; flagellum (21–26 μ). Rhizoids (2–16 μ) in diameter, richly branched, usually arising from a single point at the base of the sporangium, rarely from several points. Resting spores smooth, brown with coarsely granular contents; wall of resting spore 0.7 μ; on germination functioning as a prosporangium. Saprophytic on chitin, Van Cortlandt Park, New York City.

The life cycle and method of development of *R. chitinophilum* do not differ fundamentally from those of other species of *Rhizophydium,* so that it is not necessary to describe them in detail. Only the outstanding specific difference will be noted here. The zoospores which may remain motile up to half an hour are spherical (FIGS. 1, 2) with a long flagellum and a conspicuous refractive globule. Unlike most chytrid zoospores, however, no tail piece was found at the end of the flagellum when motile spores were fixed and stained by Couch's (**1941**) technique. In such spores a mass of deeply staining granules was persistently present (FIG. 3) which suggests the presence of a nuclear cap. The uninucleate (FIGS. 4, 5) zoospore germinates and forms a small penetration tube (FIG. 6) which by further growth and branching (FIGS. 7, 8, 9) eventually gives rise to the rhizoids, while the zoospore body enlarges and becomes the zoosporangium. The nucleus of the zoospore divides mitotically and simultaneously (FIGS. 11, 12) as the sporangium develops. After completion of mitosis and growth of the sporangium, the protoplasm cleaves progressively (FIG. 13) into uninucleate segments which become the definitive spores (FIG. 14). These ooze out in a globular mass at the time of dehiscence (FIG. 15) and disperse in the characteristic *Rhizophydium* manner (FIG. 16).



FIGS. 1–20.

The rhizoids usually arise at one point at the base of the sporangium, but in rare instances (FIG. 16) they may arise at two or several points. Like those of *R. macrosporum* Karling (**1938**) they may be unusually coarse (2–16 μ diameter), richly branched and extend for a distance of 115 μ (FIG. 14).

Resting spores of this species develop in great numbers on strips of chitin. They develop in the same manner as the zoosporangia up to a certain stage, and apparently remain uninucleate (FIG. 17) until germination begins. As noted in the diagnosis above, they are characterized chiefly by a smooth brown wall and, unlike most *Rhizophydium* species, have a coarsely and evenly granular content (FIG. 18). They germinate readily under laboratory conditions and give rise to a thin-walled, superficial zoosporangium (FIGS. 19, 20).

SUMMARY

Rhizophydium chitinophilum is a new chitinophilic chytrid which was isolated from soil from Van Cortlandt Park, New York, N. Y. This species is characterized by large, hyaline sporangia of spherical, oval, or pyriform shape, by spherical zoospores, and by smooth, brown, coarsely granular resting spores, and appears to be limited in occurrence to chitinous substrata.

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EXPLANATION OF FIGURES

FIGS. 1–20. *Rhizophydium chitinophilum.* FIGS. 1, 2. Motile zoospores, × 1200. FIG. 3. Zoospore stained with Couch's technique, × 4000. FIG. 4. Zoospore which has lost its flagellum, × 1200. FIG. 5. Zoospore germinat-

ing on chitin. Stained section, × 4000. FIG. 6. Zoospore germinating in charcoal water, × 2120. FIG. 7. Zoospore germinating on chitin agar, × 1200. FIGS. 8, 9. Developmental stages of sporangia on chitin agar, × 1200. FIG. 10. Multinucleate sporangium, × 1600. FIG. 11. Sporangium with dividing nuclei in metaphase, × 1600. FIG. 12. Sporangia with dividing nuclei in anaphase, × 1600. FIG. 13. Portion of section of sporangium cleaving into segments, × 800. FIG. 14. Mature sporangium, × 73. FIG. 15. Sporangium beginning to discharge spores, × 166. FIG. 16. Sporangium in late stage of spore discharge, × 333. FIG. 17. Stained section of resting spore, × 1500. FIG. 18. Resting spores, × 1600. FIG. 19. Germinated resting spore with attached sporangium full of zoospores, × 1200. FIG. 20. Germinated resting spore with attached empty sporangium, × 1200.

TWO ADDITIONS TO THE FUNGI IMPERFECTI

Flora G. Pollack

(with 1 figure)

Two imperfect fungi which came to the writer's attention as interceptions by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, proved to be new and are here described.

On February 8, 1946, plant quarantine inspectors intercepted in the cargo of an American steamer from China a portion of a dried tuber of *Colocasia esculenta*. Although the material appeared to be in a state of advanced desiccation, the fungus on it grew readily when transferred to corn-meal and malt agars. On corn meal it produced circular to irregular colonies which were white and cottony, with growth which was not heavy or dense. Within a week the fungus fruited abundantly over the surface of the colony, producing at length long chains of conidia which in mass were "dark greenish olive" to "olivaceous black." [1] Conidia size, shape, and color in culture were identical with that observed on the original specimen.

The fungus was tentatively determined as *Dendryphium* sp. An examination of the species of *Dendryphium* in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering revealed the presence of a culture of *Dendryphium* sp. determined by V. K. Charles and A. E. Jenkins in 1920 which had been isolated from dead cotton roots in Texas by J. J. Taubenhaus. This specimen is identical with the fungus on *Colocasia*. A third collection of the fungus was made in April, 1946, on Irish potato tuber intercepted from Mexico. This hyphomycete is here described as new.

Dendryphium obstipum sp. nov. Fig. 1, A and B

Mycelium byssoideum, subtile; hyphae 1.3 μ crassae, hyalinae; conidiophora hyalina, ad apicem subfusca, continua vel septata, 15–27 × 2.7–4.5 μ, sim-

[1] Names of colors in quotation marks are according to Color Standards and Color Nomenclature, by Robert Ridgway (1912).

plicia vel e ramis adversis composita; conidia basipetalia, juvenilissima in apice conidiophori, conidia affixa restantia, in catenis longis, viridi-olivacea, 1–3 septata, typice 3, ad septa interdum constricta, obclavata, basi rotundato ex apice acuminato, interdum hilo distincto praedita, recta vel sub apicem obstipa, 15–19 × 4–7 μ.[2]

Mycelium cottony, fine, hyphae 1.3 μ wide, giving rise to conidiophores which are flask-shaped or elongate phialides 15–27 × 2.7–4.5 μ, and may arise as simple aerial branches which are lateral or terminal, or at other times branch profusely to form candelabra-like clusters with opposite branches, the apical cells of which function as phialides. They are hyaline or slightly dark at the conidia-producing tip and when young are filled with granular protoplasm. With age they become septate and eventually are completely emptied of their protoplasm content. Conidia are abstricted from the tips of the phialides in basipetal succession, conidia remaining attached to each other, forming long chains, many of 30 or more conidia. Conidia are subhyaline when immature, becoming greenish-olivaceous with age, 15–19 × 4–7 μ, 1–3 septate, typically 3, somewhat constricted at the septa, obclavate with rounded base and pointed apex, the apex sometimes showing a distinct hilum, straight or more usually with a bend (slight to about 90°) close to the apex, which is apparently the result of continued growth of the conidia after differentiation. The conidia being joined at both ends, the apical end to a more mature conidium and the basal end to a younger conidium or conidiophore, growth is possible only in a horizontal plane, resulting in a lateral extension of the conidia as seen by the growth to one side.

On dried tuber of *Colocasia esculenta* Schott (Araceae), China (San Francisco interception 20143), February 8, 1946. On dead roots of *Gossypium hirsutum* Linn. (Malvaceae), Texas, J. J. Taubenhaus, December 1920. On dried tuber of *Solanum tuberosum* Linn. (Solanaceae), Mexico (Brownsville interception 60867), April 13, 1946.

Culture on corn-meal agar from *Colocasia esculenta*. **Type** in Mycological Collections *71499*.[3]

The second fungus, a new species of *Bartalinia* on *Nolina microcarpa*, was intercepted at Nogales, Arizona, on May 26, 1945.

[2] The writer wishes to acknowledge the help of Miss Edith K. Cash in the preparation of the Latin diagnoses in this article.

[3] The specimens cited in this paper have been deposited in the Mycological Collections of the U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

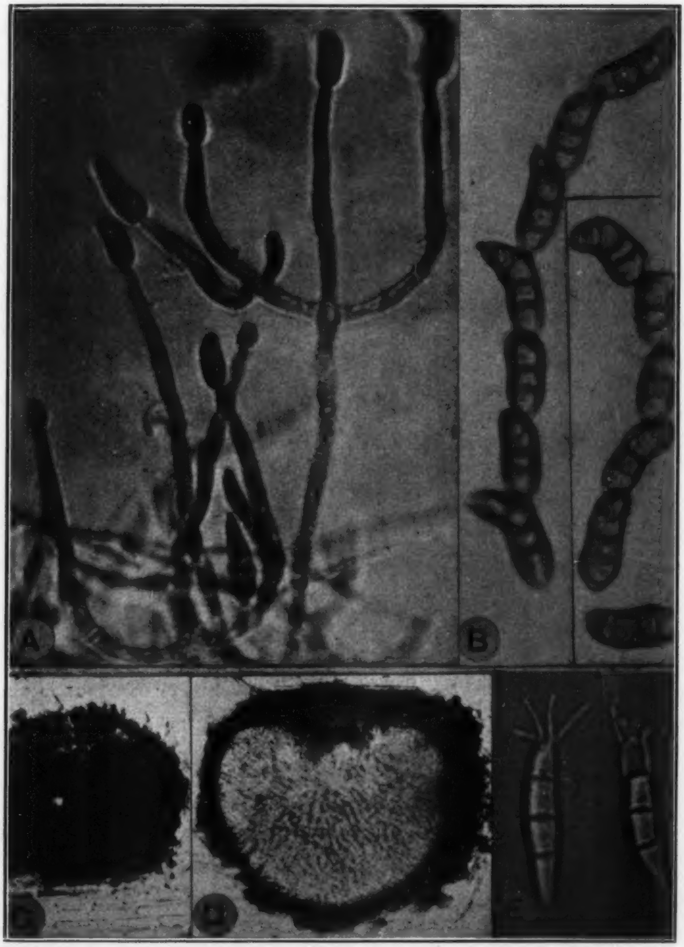

FIG. 1, A and B. *Dendryphium obstipum* from culture. A, Conidiophores showing candelabra-like branching, × 1000. B, Chains of conidia, × 1000. C–E. *Bartalinia nolinae*. C, Top view of a pycnidium, showing ostiole, × 100. D, Cross-section through a pycnidium, × 100. E, Unstained conidia showing setae and inconspicuous basal mucous plug, × 570.

Bartalinia nolinae sp. nov. FIG. 1, C–E

Pycnidiis globosis vel subglobosis, usque ad 400 μ in diam., ostiolatis, subepidermalibus; conidiis clavatis, hyalinis, 2–3-septatis, rectis vel subcurvatis, 36–54 × 6.8–9.5 μ (setis et obturamento basali mucoso exclusis), apice setis tenuibus 2–7, plerumque 3–4, interdum ramosis, 6–24 μ longis, et obturamento basali mucoso 3–5 × 3 μ munitis; conidiophoris ellipsoideis usque subulatis, 6–15 × 2–4 μ.

Pycnidia formed in between the leaf veins, globose to subglobose, up to 400 μ in diameter, with a dark pseudoparenchymatous wall, 24–60 μ thick, ostiolate, innate, subepidermal, the pycnidia lifting out easily from the dried disintegrating tissue of the host. Conidia clavate, basal end rounded, somewhat narrower than the rounded to obtuse apical end, 2–3 septate (usually 3), not constricted at the septa, straight or slightly curved, hyaline, 36–54 × 6.8–9.5 μ (excluding setae and basal mucous plug). Setae arising from different positions on the upper surface of the apical cell, 2–7 (usually 3 or 4), delicate, sometimes branched, 6–24 μ long, and mucus-like plug at the basal end, inconspicuous, 3–5 × 3 μ. The setae, basal mucous plug, and spore wall do not take such stains as phloxine or cotton blue in lactophenol. Conidiophores short, ellipsoid to subulate, 6–15 × 2–4 μ, hyaline, occasionally adhering to conidia discharged from pycnidia.

On dead leaves of *Nolina microcarpa* S. Wats. (Liliaceae). Vicinity of Nogales, Arizona (Nogales interception 62345), May 26, 1945, P. R. Frink. **Type,** in Mycological Collections *71498.*

Saccardo set up *Pestalozzina* as a subgenus of *Pestalozzia* (now *Pestalotia*) with the note that it was identical with *Pestalozzia* except for the hyaline conidia. In recent years Guba (**1**), Wolf (**3**), and others have broadened the concept of *Pestalotia* so that it covers fungi of the *Pestalotia* type which produce conidia in either acervuli or pycnidia. No comparable study has been made of *Pestalozzina.* Although it seems probable that such a study might lead to a broadening of the genus, until it is made, only melanconiaceous fungi can be assigned to the genus *Pestalozzina.* The genus *Bartalinia* (**2**) which was set up for hyaline conidia of the *Pestalotia* type in pycnidia, will have to be retained for the time being.

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NOTES ON THE GENUS ARMILLARIA

Alexander H. Smith [1] and Maurice B. Walters

(with 1 figure)

Armillaria decorosa (Pk.) comb. nov. (fig. 1)

Agaricus (*Tricholoma*) *decorosus* Peck, Bull. Buffalo Soc. Nat. Sci. **1**: 42. 1873.
Tricholoma decorosum Saccardo, Syll. Fung. **5**: 111. 1887.
Cortinellus decorosus Murrill, North Amer. Flora **10**: 32. 1914.
Tricholomopsis decorosa Singer, Mycologia **35**: 152. 1943.

This very rare agaric occurs in the vicinity of Cleveland, Ohio, where it is usually found limited to three or four fruiting bodies at the most. It appears rather late in the year, from October to mid-November. The exact dates on which it has been found during the past twelve years are November 16, 1935; October 14, 1938; October 19, 1941; and September 30, 1945. It has always been found growing on well-decayed and usually well-soaked logs of deciduous trees in a beech-maple-hemlock forest.

This species is perfectly illustrated in Icones Farlowianae, pl. 17, as *T. decorosum,* by specimens whose identity was confirmed by Peck himself. The Ohio material checks almost perfectly with the account in the Icones and so we have limited our comments here to a few on the fruiting bodies instead of giving a complete technical description. The pileus after expanding from the semiglobose shape of the young stage sometimes reached a diameter of 6 cm. It is cinnamon brown in color (Maerz & Paul 14–K–10), somewhat darker toward the center and thickly covered with long, fibrillose, erect scales of a darker color (blackish at tips). The margin of the pileus extends well beyond the gills. The gills are white, close, adnexed, broadest near the stipe, rather finely serrulate, and sometimes vertically costate. The stipe is sheathed up to the annulus with a coating similar in color and texture to that

[1] Papers from the Department of Botany and the University of Michigan Herbarium.

FIG. 1. *Armillaria decorosa.* ×1. Photo by M. B. W.

of the pileus. It is smooth above the annulus, and usually tapers upward from a somewhat clavate base. The spores are smooth, elliptic, $6 \times 4\ \mu$, and amyloid.

Just why Peck considered this a *Tricholoma* rather than an *Armillaria* is not clear. In the Friesian system, which was the one followed by Peck, it would seem to be a much better *Armillaria*. It would certainly fall within the concept maintained by Kauffman (Pap. Mich. Acad. Sci. **2**: 53–67. 1923), since that concept is broader than that of Fries to the extent that the granulose species, which were placed in *Lepiota* in the Friesian system, were transferred to *Armillaria*. These granulose species have been recognized as a distinct genus and monographed under the name *Cystoderma* (Smith & Singer, Pap. Mich. Acad. Sci. **30**: 71–124. 1945). *Cystoderma* contains certain lignicolous agarics very similar in aspect and fundamental characters to *A. decorosa*, but differing in having the cap surface covered by sphaerocysts instead of true filaments. This one character is all that prevents *A. decorosa* from being classified as a *Cystoderma*, and seems to us to indicate a close relationship between the two genera. Singer (*l.c.*) transferred *A. decorosa* to his genus *Tricholomopsis*. He described the spores as non-amyloid. We tested the spores of the Ohio collections now preserved in the Univ. of Michigan Herbarium as well as one collection by Kauffman from the Adirondack Mountains in New York, and found them to be amyloid. Dr. Singer very kindly rechecked the material at the Farlow Herbarium for this character and reported that this time he obtained a weak amyloid reaction. On this basis the fungus should not be regarded as congeneric with *Tricholoma rutilans*, and hence we exclude it from *Tricholomopsis*. In our estimation it appears to be closely related to *Armillaria luteovirens*, the species proposed as the lectotype of the genus by Singer and Smith (Mycologia **38**: 259. 1946), both because of the presence of clamp connections and amyloid spores as well as the type of veil and the distribution of its remnants. Since in the closely related genus *Cystoderma* the habitat may be either lignicolous or terrestrial, that character cannot be given much emphasis here although it might be mentioned in passing that the lignicolous habitat is quite atypical as far as *Tricholoma* is concerned.

ARMILLARIA LUTEOVIRENS **f. alba** A. H. Smith f. nov.

Pileus 3–6 (8) cm. latus, albus, squamosus demum levis; lamellae albae, adnatae vel emarginatae; stipes 4–9 cm. longus, 5–10 mm. crassus, deorsum alba-squamosus, sursum glaber et albus; sporae 6.5–8 × 4–5 μ, amyloideae. Specimen typicum A. H. Smith n. *20267;* in Herb. Univ. Michigan conservatum; legit prope Crown Point, Columbia Gorge, Ore., Oct. 30, 1944.

Pileus 3–6 (8) cm. broad, obtusely conic to convex becoming broadly umbonate to plane, surface moist, at first innately squamose with long, flat scales which usually are recurved at the tip, more or less glabrescent in extreme age, dull white over all; flesh white, soft, thick in disc, odor and taste not distinctive; lamellae close, moderately broad, adnate becoming emarginate, edges even; stipe 4–9 cm. long, 5–7 mm. thick at apex, evenly enlarged downward, dull white throughout, with a superior submembranous annulus, silky and smooth above the ring, coarsely squamose below from innate, long, flat, recurved scales, somewhat glabrescent in age, spores 6.5–8 × 4–5 μ, ellipsoid to oblong, weakly amyloid, smooth; basidia four-spored; pleurocystidia and cheilocystidia none seen; gill trama parallel; pileus trama homogeneous or cells over surface somewhat collapsed and subgelatinous in age, clamp connections present.

This white form was found growing with typical specimens by Wm. B. Gruber in 1941; and by both Gruber and Smith in the same area again in 1944. This is apparently the first record of this species for North America.

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